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CHANGES IN NORMAL ELECTROENCEPHALOGRAM OF *MACACA MULATTA* WITH GROWTH*

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(Received for publication May 7, 1942)

INTRODUCTION

THE CORTICAL POTENTIALS of the human infant have been shown by a number of investigators (1, 2, 3, 6, 7, 8, 9, 10) to change with increasing age. In the present investigation similar changes were found in the electroencephalogram (EEG) of the infant monkey (*Macaca mulatta*). The problem was undertaken at this time because the EEG of monkeys was known to resemble that of man in many of its major characteristics, and it was felt that analysis of such cortical potentials might be of value in further interpreting the functions of the central nervous system. Since the period from birth to maturity in the macaque occurs in the relatively short space of four years, the age of the animal and the expected pattern at a given age are significant in the analysis of normal and abnormal EEGs.

METHOD

A Grass, 3-channel ink-writing oscillograph was used.† Solder electrodes 4 mm. in diameter moistened with electrode paste were attached to the shaved skin of the head with collodion. Six positions were used—left and right frontal, parietal and occipital areas (Fig. 1). Indifferent electrodes were attached to each ear (E). Records were usually made in four arrangements of electrodes which were supplemented by others when indicated. The usual recordings were (Fig. 1): (1) Direct leads from the left side of the cortex to Ear, 1E, 2E, 3E. (2) The same from the right, 4E, 5E, 6E. (3) Interhemisphere, 1–4, 2–5, 3–6. (4) Inter- and intra-hemisphere, 1–3, 2–5, 4–6.

FIG. 1. Diagram of monkey's head showing position and numbers of scalp (1–6) and ear (E) electrodes as used throughout the experiments.

The larger monkeys were restrained in holding boxes (Fig. 2) but the smaller infants were swaddled and held by one of the investigators throughout the procedure. The eyes were bandaged shut and the room kept in semidarkness. No anesthesia was used, but after preliminary struggling and some periodic effort the monkeys were usually sufficiently quiet so that cortical potentials could be recorded without contamination by muscle potentials. One hundred and fourteen electroencephalographic records were taken from 34 monkeys. Seventy of these were from 17 animals of known age during the first year and a half of life. The remaining 44 records were from 17 animals of known weight but the ages of which were only estimated from the size, weight and state of maturity of the individual.

* Aided by grants from Child Neurology Research (Friedsam Foundation) and Knight Fund, Yale University School of Medicine.

† EEG apparatus was made possible by the courtesy of a grant from the Josiah Macy Jr. Foundation to the Department of Neuroanatomy, Yale University School of Medicine.

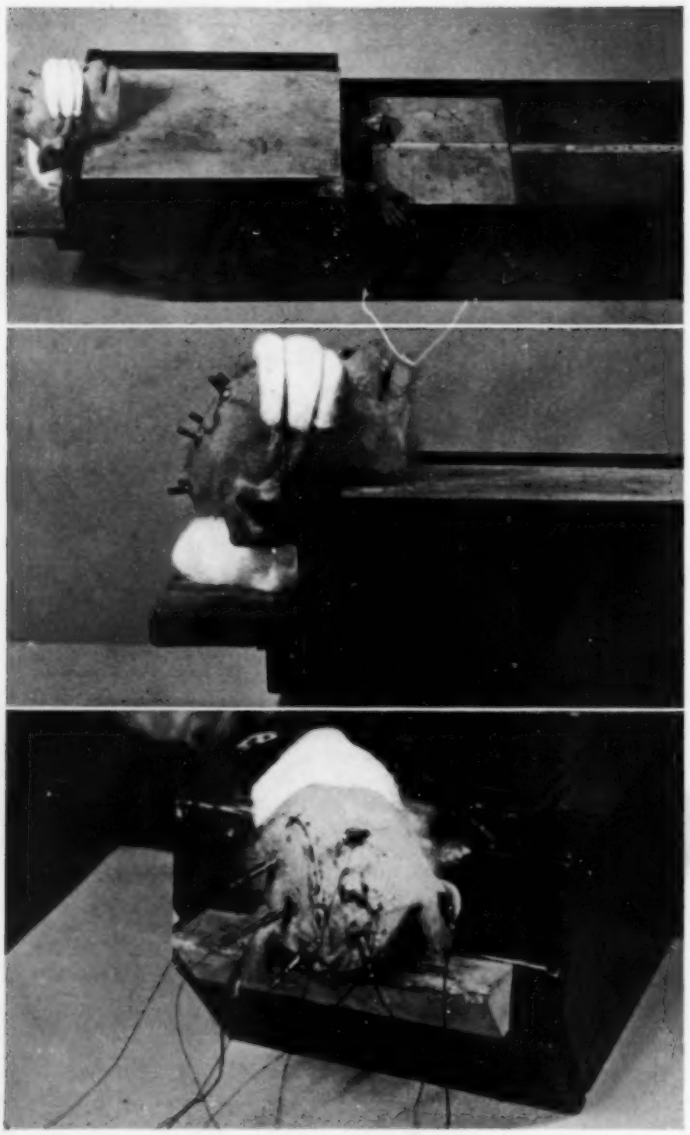

FIG. 2. Holding box for monkey. Solder electrodes and leads in place for recording EEG. The animal is conscious and in no pain.

DATA

General characteristics of normal EEG. At any age the EEG of the normal monkey has a definite pattern and characteristic features which resemble those seen in the human (Fig. 3). Although there are individual modifications of pattern and changes with age as will be shown, these can be expressed

as the measurable variables, rate and amplitude. The effect of sleep, of opening and closing the eyes, and of stimuli such as noise or light on these potential changes can be seen and accurately interpreted. Amplitude varied at different ages from 20 to about 80μV. Three frequencies, or rates per second, can be in general separated, called usually fast, medium and slow, or beta, alpha and delta (1) when used to characterize the pattern in man.

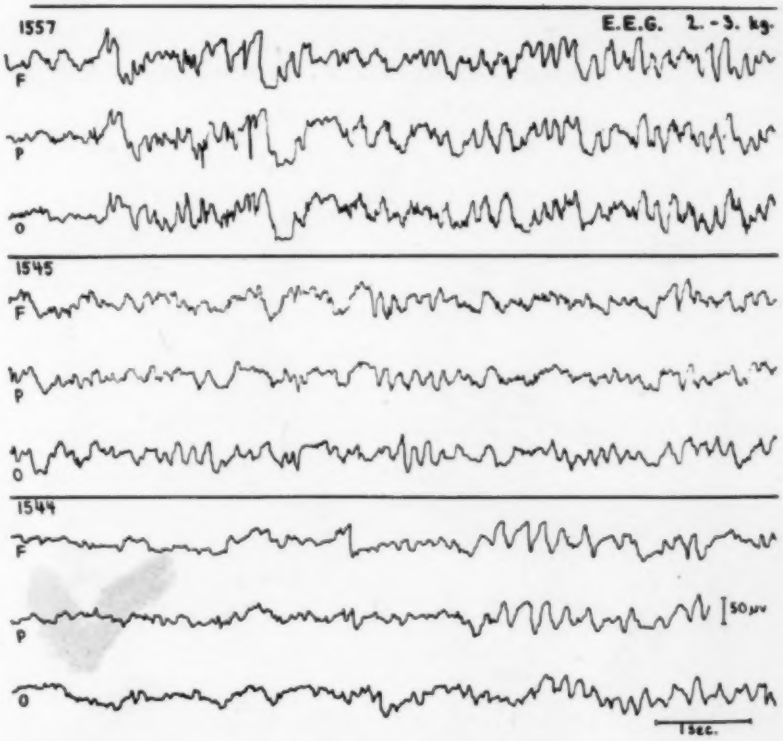

FIG. 3. Normal EEG records from 3 *Macaca mulatta* of 2–3 kg. weight from F, frontal, P, parietal, O, occipital leads. Indifferent electrodes on ear.

The most obvious waves in both man and monkey are of medium frequency varying with age in the monkey from 3–4 to 10–12 per sec.

No focal changes were noted with growth, the oscillations of potential were usually of the same rate, amplitude and direction (+ or −) at the same time within one hemisphere from frontal parietal or occipital areas. Furthermore, the intra-hemisphere oscillations were usually alike in all respects on the two sides. But potentials from interhemisphere leads were usually lower than those of intra-hemisphere recordings made simultaneously. This difference was found to be accentuated following cerebral cortical ablations (4).

During sleep, the regularity of pattern became disturbed and the amplitude increased so that large irregular oscillations would appear gradually in all leads and disappear suddenly if the animal were wakened. If records were taken from a monkey awake and unblindfolded but in the dark, there would be a characteristic "damping" of the picture when lights were turned on. Amplitudes became smaller and regularity of pattern increased. Loud sounds or focussing of the attention by any other means caused the same type of change.

EEG during first week of life. The EEG of the new-born infant monkey can be easily identified by its exceedingly low and slow potentials, and by

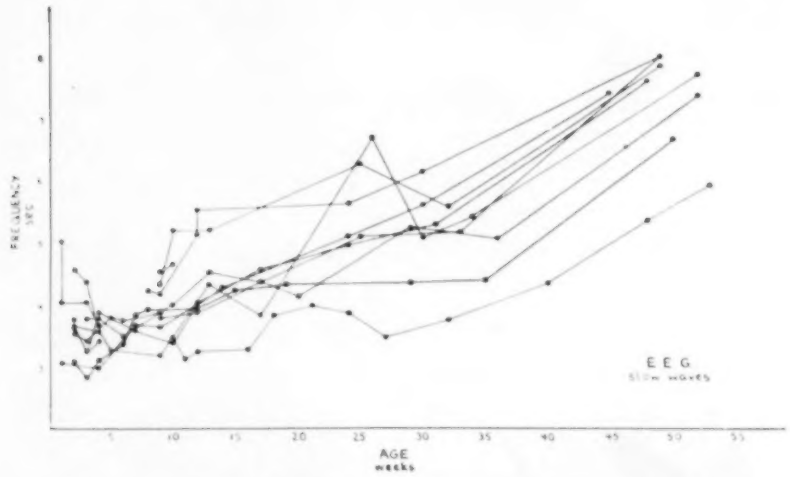

FIG. 4. Frequency of EEG waves during 1st year of life. Lines represent individual animals (*Macaca mulatta*).

the simplicity of pattern (Fig. 4) as compared with that of older animals (Fig. 3). At most there is relatively little activity but what is present varies markedly, showing, at intervals, bursts of 2–3 per sec. waves. The base-line is unstable because of long slow waves, each one lasting 3–6 seconds. There are relatively few fast oscillations.

At this age it was found impossible to correlate the potentials with sleep because the infants, during the first two months are either asleep, drowsy or struggling. When held for the EEG recording, they usually vocalized and struggled, then quickly relaxed and clung limply with eyes shut. During this time, when respirations were slow, deep and regular, it was assumed that the infant slept, but the stages in relaxation were so slight and gradual that no true state of sleeping was ever certain. In the older animals, sleep could at times be definitely ascertained. From the evidence of Smith (11) it is probable that the variability in activity of the cortex in the new-born was in part at least dependent on the state of sleep or wakefulness.

There was an individual difference in the amplitude of the potential changes in these infants under a week old, as shown in eighteen records made on 11 infants. It appeared also in 7 records made on 7 infants on the first day of life. When the records were arranged according to the weight of the infants it was found that smaller infants showed simpler records with slower amplitude than larger. Body weights varied from 355 to 515 gm. at birth. No data were available concerning exact number of days of gestation. It is possible, however, that the smaller infants were actually younger than the larger, and functionally better developed animals. Since the differences at birth were so striking and the character of all records changed most markedly during the first two or three weeks, it may be assumed that in the infant monkey relatively rapid cortical development occurs during the immediate pre- and post-natal period.

Localization of function within the various cortical regions has been found in the human infant (8) in which the region about the central sulcus first shows changes in potential. In the monkey, although this area is known to develop anatomically and functionally before either frontal or occipital poles, no very significant localization of activity appeared on records made from the surface of the head, although in a few instances the occipital leads registered less activity than did either frontal or central. It is probable that this is due to spread of current on the very small surface of the infant head, which is never larger than 6 cm. in diameter, and on which the edges of the electrodes could never be farther than about 1 cm. apart.

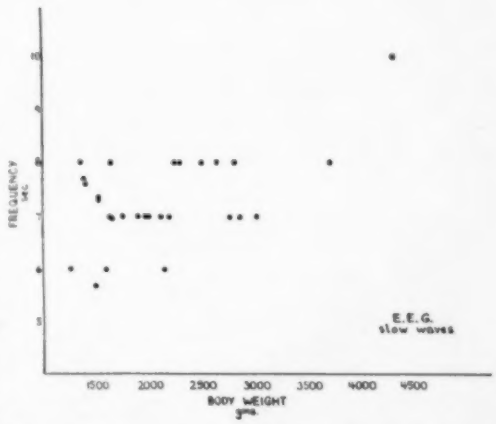

FIG. 5. Frequency of EEG waves in young and adult *Macaca mulatta* showing range of 7–10 per sec. after 2nd year (weight 2500 gm.) of life.

EEGs at four weeks. By the fourth week there were very noticeable changes in the records. Amplitude increased slightly and rate changed from about 2–3 to 4–5 per sec. (Fig. 5). The records also became more complex and less variable than those of the new-born. The wavering base-line producing slow waves of 2–3 sec. frequency was less often present. Such changes appeared occasionally in the records of the two-week-old infants, in others slightly later. In all, the most distinct and rapid changes which appeared at any time took place before the end of the first month.

At this time variability still persisted and a type of wave which gradually disappeared during the latter part of the first six months was very

noticeable. This was seen as infrequent bursts of very rapid waves, occurring spontaneously and not related to any known state of the animal. They lasted only 2–3 sec. at a time and the rate was about 15–20 per sec. They were not unlike the episodes which we have observed after cerebral ablations in older animals (4). At this age sleep and waking could sometimes be distinguished. During sleep the medium frequency waves were accentuated.

EEGs from 2d to 6th month. By the second month amplitude of the waves had increased greatly, often to the maximum for the particular animal. There was by then no difference in occipital, frontal or parietal records. Am-

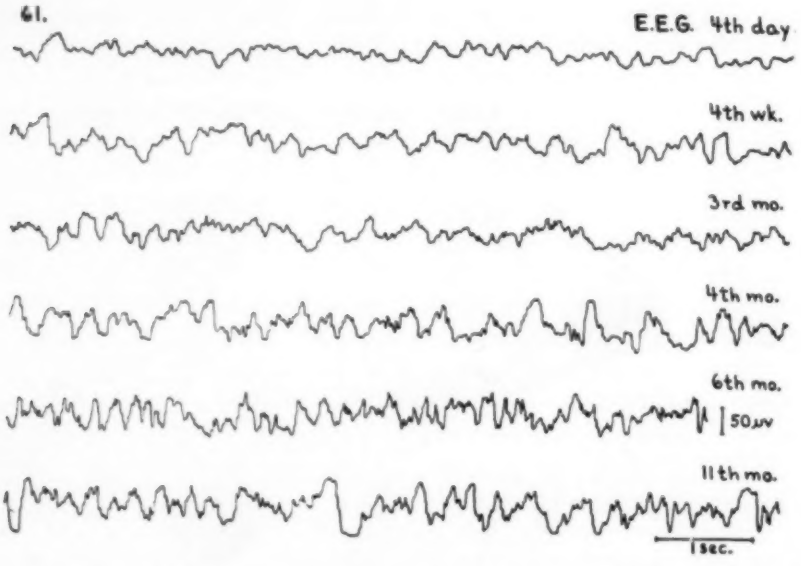

FIG. 6. EEG records at intervals during 1st year of life of a *Macaca mulatta*. With increasing age amplitude, rate and complexity of pattern are increased.

plitude by the fourth month was always maximal and it then often decreased slightly during the remainder of the first six months of life. Frequency increased (Fig. 6) to a rate of 5–6 per sec. by the fourth month and as high as 6–7 per sec. by the sixth month.

EEGs from 6th to 12th month. During the second half of the first year of life records were taken monthly from two animals of known age and from four others of about the same weight (1700–2000 gm.). In this age group frequencies of the most obvious waves had increased to between 6 and 8 per sec. (Fig. 6). Amplitudes, in contrast, had decreased. Throughout the record of one individual on a single day or for many days, there was less variability in the type of potential changes than in the younger infants. The wavering base-line had almost entirely disappeared but the medium and fast rhythms formed complexes as intricate as those seen in the adult animals.

Sleep could not be distinguished from waking though there were intermediate stages. The flattening of the waves which appeared with awakening could be made more pronounced by external stimuli such as loud sound, opening of the eyes or a bright light. Mere focussing of the attention to noise, etc., seemed to produce this flattening of the waves and decrease in amplitude.

EEGs of monkeys weighing from 2000–2500 gm. The largest number of older monkeys on which records were made lay in this group (17 records on 8 animals) which consisted of animals probably in the middle of the second year of life. Because the rate had now increased to about 8 per sec. and amplitude was decreased, many of the smaller variations seemed to be ironed out (Fig. 3). These records were not now very different from those of the adults.

EEGs above 2500 gm. weight. All the records from older animals (14 records on 7 monkeys) were characterized by low amplitude and rapid rate which usually reached 10, and occasionally 12 per sec. was comparable with the alpha rhythm of the human. These records were very uniform but different animals showed individual characteristics.

The reason for the marked decrease in amplitude of the waves was possibly, as in man, due to increase in thickness of the skull. This has been found to materially affect size of recorded potentials. But, records in a few instances were made directly from the surface of the brain of infants and of adults and in these cases also the character of the two ages remained different. Those of the infant showed widely variable potential changes with great fluctuation in amplitude and rate, while those of the adults had more even and regular fluctuations with more rapid rate and lower amplitude.

Discussion

From these data it seems that the evolution of the adult EEG in the young monkey has much in common with similar changes which occur during the same process in man. In each, the picture becomes stabilized to the adult level at a relatively early age; in man at 12 years and in the monkey during the second year; well before maturity of the entire organism. In each, the most pronounced changes occur very early; in man during the first year and in the monkey during the first month. In both the changes with age are those of increase in frequency of potential waves and of an early increase in amplitude followed by a later and slighter decrease in the latter.

The changes in EEG have been correlated in man with the anatomical and physiological development of the central nervous system and may be so correlated in the monkey. Lindsley (7), quoting Weinbach, says that cortical potential changes can be correlated with growth of brain in man. This correlation is about as good in the infant monkey for in these animals, weight of brain increases largely during the first month and little after the second year of life (5).

The development of motor function in the monkey also occurs largely during the period of most marked change in EEG, *i.e.* the first two months of life. During this time the infant monkeys change from a state in which they are able only to right, climb and grasp reflexly to one in which there are responses to visual and auditory stimuli and in which walking, climbing and the beginnings of voluntary prehension appear. By the end of the first 6 months infants are capable of an adult type of motor performance. All evidence therefore points to the fact that with the development of cortical integration of motor power there is a coincident change in electrical potentials and that this occurs at the time of maximal anatomical and functional differentiation of the cerebral cortex.

The electroencephalogram of the adult monkey has been shown to be similar in many characteristics to that of man. That there is not, however, as much localization of function within different areas of the cortex might be expected because of the relatively small size of the monkey brain, and of the relative lack of complexity in its organization.

Conclusions

1. In the infant monkey cortical potentials (EEG) begin to develop at or before birth, but are not well demarcated until three or four weeks after birth.

2. From this time until the end of the sixth month there is progressive development and elaboration of the EEG until it resembles that of the adult.

3. Frequency of waves is about 2–3 per sec. immediately after birth. It increases to about 7–8 per sec. during the first six months and then slowly to about 10–12 per sec. by the end of the second year.

4. Amplitude is low at birth, increases during the first six months and then slightly decreases.

5. During the growth period the EEG becomes at once more complex and more uniform. There are less variations of base-line and of type of potential.

6. The effect of sleep can be detected in the older animals, but in the newborn infant fluctuations in state of waking or sleeping are too slight and gradual to be correlated with the marked fluctuations in type of EEG which may however be related.

7. The changes in the development of the infant monkey are like those described for man and are as far as is known coincident with the anatomical and functional development of the cerebral cortex.

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EFFECT ON ELECTROENCEPHALOGRAM OF LESIONS OF CEREBRAL CORTEX AND BASAL GANGLIA IN *MACACA MULATTA**

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(Received for publication May 7, 1942)

INTRODUCTION

WITHIN the last five years the electroencephalogram (EEG) has become recognized as a useful means of diagnosis of specific lesions in the central nervous system in man. Abnormal foci such as tumors (23, 21) can often be detected and the changes which result from epilepsy (6, 7, 8, 13, 21) or from increased intracranial pressure (5) are now easily recognized. The monkey, under normal circumstances has an EEG which is comparable in many respects to that of man (17). In the present article the effects on EEG of focal lesions of the central nervous system of such animals are described.

METHOD

Lesions have been made in various portions of the *cerebral cortex* and *basal ganglia* of 41 monkeys (*Macaca mulatta* or *Cercocebus torquatus atys*) and EEGs taken before and after operation have been compared. Both acute and chronic experiments were carried out. In each, the lesions were made by the same operator and the same technique. The acute experiments were executed under dial anesthesia (0.3 mg. per kg. given, ½ intraperitoneally and ½ intramuscularly) which ensured a long and even state of anesthesia. Operations for the chronic preparations were done under sodium amytal and with aseptic precautions. Except in the acute experiments, records were made on unanesthetized animals restrained as shown in the preceding article (17). In nearly every instance, after a certain amount of struggling the animals relaxed and permitted the recording of cortical potentials uncontaminated by those of muscular activity. The subjects were blindfold and in semidarkness in a quiet room.

A Grass, three-channel, ink-writing oscillograph was used.† Six solder electrodes were applied with collodion to the shaved skin of the head, over the frontal, parietal, and occiptal regions in the six positions described in text and figures of preceding article (17). The same combinations of electrodes were also routinely recorded and supplemented by others whenever indicated.

EEG records were made on chronic preparations of two types, namely, those operated on in early infancy and older animals. The two groups will be discussed separately as results show that, as is known in human cases, the effect on the EEG of lesions sustained before either anatomical or physiological development is complete may be quite different from that on the EEG of a mature brain.

DATA

A study of the preoperative records of the monkeys of this series shows normal EEG patterns such as have been described previously. The varia-

* Aided by grants from Fluid Research Funds of Yale University School of Medicine and Child Neurology Research (Friedsam Foundation).

† EEG apparatus was made possible by the courtesy of a grant from the Josiah Macy Jr. Foundation to the Department of Neuroanatomy, Yale University School of Medicine.

tions due to age, sleep, light, sound or other factors of attention, or to individual peculiarities could be recognized, and hence, new characteristics appearing after cerebral ablations could be detected.

CONTROLS

The effects on EEG of elevation of skin, bone and dural flaps was tried on three animals. In the first, the skin flap alone was opened and closed, in the second, skin and bone flap, and in the third, the dural flap was opened and the cortex left exposed to the air for ten minutes but without being touched. EEGs measured on the days following these procedures showed no change in rate and none in pattern except that the amplitude was slightly diminished for a day or two at most. It was thought that this latter was due to scalp edema which increased the distance between the active cortex and the recording electrodes. Later, cortical lesions in these same animals produced the characteristic changes in potentials described below.

ACUTE EXPERIMENTS

Under dial anesthesia and with leads placed directly on various areas of cortex, records were taken from three animals. No effect on EEG was observed from any lesion made either in cortex or basal ganglia by this means. In two cases portions of cortex were removed by suction successively from areas 4, 6, and the postcentral gyrus, followed by lesions in caudate and putamen. In one of these the frontal and occipital lobes were later extirpated. No effect on EEG developed as recorded from islands of tissue left intact in area 4.

The third animal's cortex was left undisturbed and the caudate was removed via corpus callosum first from one and then both sides. Later, the putamen was destroyed through a small hole in area 8. There was no change in EEG. As will be discussed later, similar lesions in chronic preparations caused marked changes in EEG. The lack of such changes here can be attributed either to the acute state of the preparation or to the anesthetic.

CHRONIC EXPERIMENTS

A. Non-specific effects of lesions of central nervous system occurring during first postoperative weeks. Immediately following injury to the central nervous system, *i.e.*, during the first one to ten days, EEGs were altered materially and in the same way no matter where the cerebral lesion had been made. The size of the lesion, however, affected the duration of these non-specific changes, as small lesions produced effects lasting only a day or two, while the effects of larger ablations might be visible for more than a week.

The records during such a period as compared with those preceding operation were lower in amplitude and slower in rate. The changes in amplitude, as in the controls, could be due to scalp edema, but, in addition, the rate of the 8 per sec. waves was *often* slowed as much as 2–3 cycles and *usually* 1–2 cycles per sec. Moreover, during this period, the fast component became less obvious so that the entire record appeared even, regular, slow

and smooth as compared to the more variable and rapid oscillations in the preoperative picture (Fig. 1).

Occasionally such changes did not appear on the first day after operation, but on the second, and became more pronounced on the third and fourth, gradually diminishing after that date. The time sequence follows closely that previously observed on a large series of animals of the reaction of tissue to injury. Cerebral edema is usually worst on the second postoperative day,

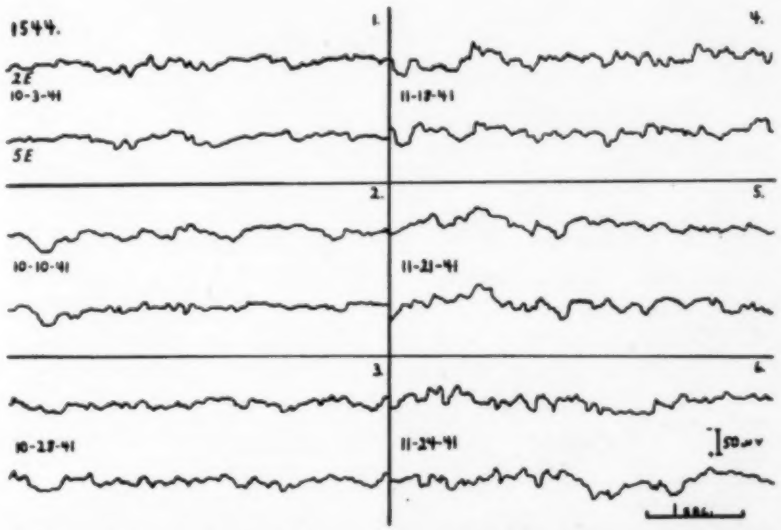

FIG. 1. Record from a large *Macaca mulatta* (3–4 kg.): 1, normal; 2, After "control" reflection of skin and bone flap only on Oct. 6, 1941; 3, and 4, After ablation of left areas 4 and 6 on Oct. 27, 1941; 5, and 6, After removal of entire left hemisphere on Nov. 19, 1941. Records are from two sides of head (2E left and 5E right parietal).

and severe only for three to four days. It may subside during the ensuing week.

B. Focal effects of cortical lesions. During this series of experiments, pre- and postoperative EEG records have been made from monkeys with bilateral and unilateral lesions of every area of the cerebral cortex. The majority have had lesions of the sensorimotor cortex, in particular of the motor areas in the frontal lobe. In many cases these were combined with subcortical lesions in the caudate and putamen.

1. Hemispherectomy (4 monkeys). When the entire cerebral cortex was removed from one hemisphere leaving basal ganglia uninjured, no characteristic changes in EEG (Fig. 1) pattern resulted. After the initial non-specific changes, the picture returned to that which preceded operation, and in two such instances, has remained so for more than three years after operation. The other two animals which were kept alive for shorter periods likewise showed no changes. Leads placed in the usual positions recorded patterns which were identical for the two halves of the skull, except that those lying

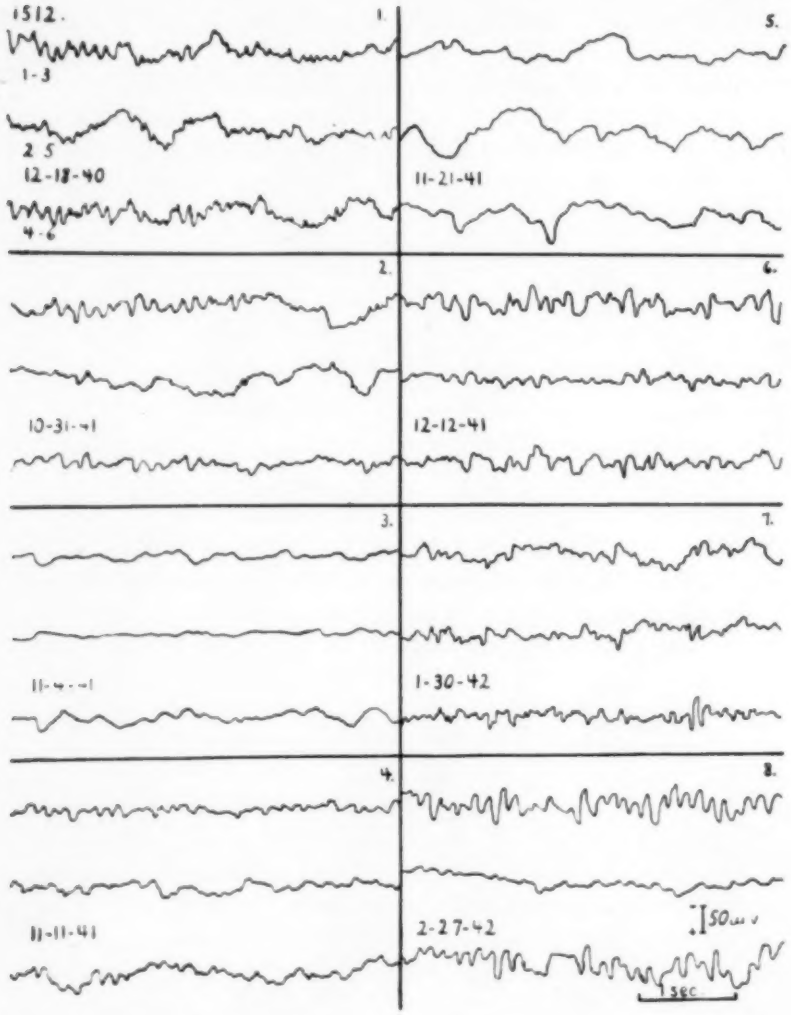

FIG. 2. Electroencephalograms from an immature *Macaca mulatta* (2.5–3.5 kg.) showing: 1, and 2, Normal pattern; 3, Effect of ablation of left area 6 on Nov. 3, 1941; 4, Recovery; 5, Effect of ablation of right area 6 on Nov. 18, 1941; Followed by return to preoperative pattern (6, 7, 8).

above the active tissue were slightly greater in amplitude than those above the ablation. It is to be remembered that the diameter of these monkeys' heads is 4–6 cm. and that the individual leads are never more than 2 cm. apart, a factor which makes localization less possible than on the human head.

The fine, rapid components of the EEG and the slower 8–10 sec. waves were unchanged after the first week. There was also as much variability in the pattern as had been present before operation. The effects of light, sleep, sound, etc., were as easily visible.

2. Lesions of sensorimotor cortex (9 monkeys). Neither unilateral nor bilateral ablations from any part or parts of the sensorimotor cortex produced any very significant change in EEG in the older animals (Fig. 2). It was noted several times that the postoperative records showed a greater tendency to bursts of deep regular 8 per sec. waves called hypersynchrony by Jasper (11, 13), but reexamination of all records did not show that there was anything more than a suggestion that these irregularities were more frequent after than before operation. In the infant records, as will be seen later, this tendency became much more definite.

Unilateral and bilateral ablations were made as follows: Area 6, two monkeys; area 4, one monkey; areas 4 and 6, four monkeys; postcentral gyrus, two monkeys; and angular gyrus, two monkeys. Each lesion was followed by the expected clinical picture characteristic for the areas injured. In some instances records have been taken at intervals for as long as two years after operation.

3. Lesions of frontal association areas (4 monkeys). Pre- and postoperative records from 4 animals which had had extirpations of areas 8–12, the cortex lying rostral to the motor areas, showed no significant changes in pattern and no localization of lesion. These animals after bilateral ablations developed the changes in behavior and greatly increased activity which are characteristic of removal of these areas and which are considered by some to indicate "release of function" of one type just as spasticity is assumed to be another evidence of a release mechanism. In neither instance did EEGs show release of function.

4. Lesions of temporal and occipital lobes (5 monkeys). Postoperative records after bilateral occipital lobectomy in one animal (made two years previously), revealed nothing unusual except that there was no response in EEG to visual stimuli of light and dark. The monkey had, clinically, no vision but had shown response of pupillary constriction to light. Records were made on four animals before and after temporal lobectomies. There were no changes in the character of the EEG.

5. Cortical ablations made in infancy (8 monkeys). As reported previously (17) EEGs of normal infant monkeys have been made from birth until 2 years of age. Seventeen such infants had ablations from the central nervous system during early infancy. Eight of these were in cortex only and with 3 exceptions these eight, like the older animals showed no change in EEG after the first generalized reaction.

The three exceptions were monkeys which had had all of areas 4 and 6 removed bilaterally. After the initial slowing and flattening phase of the waves the pattern returned to normal for some months. In each case however, there *later* developed a slight but definite change which was like that found following lesions of cortex and basal ganglia combined, the characteristics of which will be described below. There are two possible explanations for this; either large cortical lesions of areas 4 and 6 made in infancy will eventually produce this alteration by rearrangement of cortex itself, or, as

the brain develops and grows, cortico-subcortical relationships are altered as they are in older animals with cortico-subcortical lesions.

C. Effects of lesions of caudate and putamen (4 monkeys). In contrast to the minimal effects of pure cortical lesions, ablation of head of caudate nucleus or of caudate and putamen produced very marked changes (Fig. 3). These changes were observed immediately after both unilateral and bilateral extirpations in 2 infants and 2 adults. Those produced in the infants were much greater than those in the adults, but of the same type. Figure 4 shows

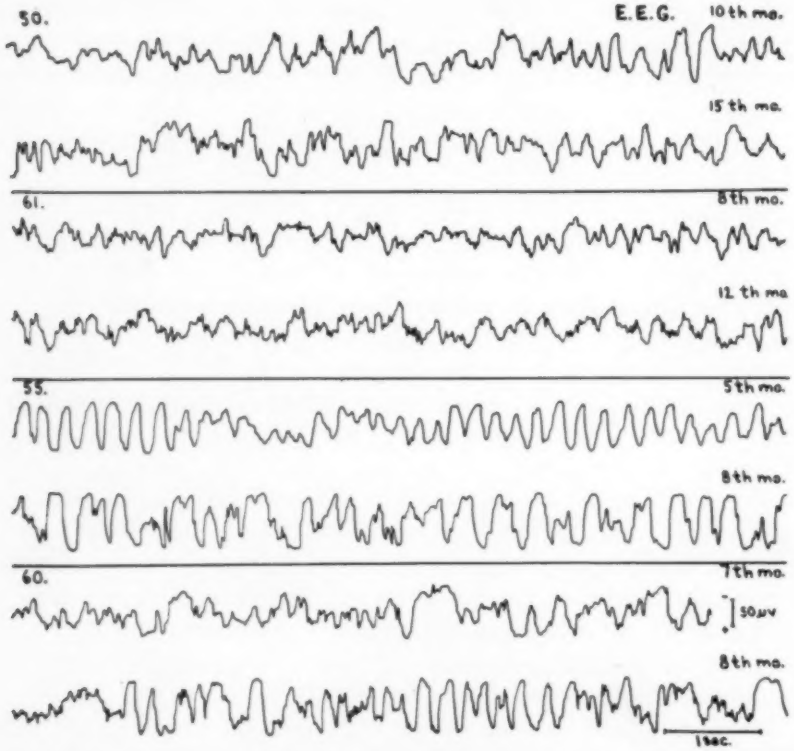

FIG. 3. Postoperative records from 4 young *Macaca mulatta*, the upper two (50 and 61) show nothing abnormal in EEG following cortical ablations (areas 4 and 6, bilaterally); the lower two (55 and 60) show marked hypersynchrony following bilateral ablations of areas 6 and caudate nucleus.

samples of the records from one such case. This macaque was born in the colony. EEGs taken on the fourth day and during the fourth week of life showed normal rate and amplitude for an infant at those ages. Fast and medium waves were rather variable as is normal at this time and there were occasional slow waves at about 2–3 per sec. superimposed on the 4–5 per sec. faster rate.

During the fourth week of life the left caudate nucleus was removed by suction via a small hole in area 8. Two days after operation when the next

record was taken there was a change in the character of the potentials. This persisted and became intensified after removal of the second caudate during the sixth month. For the succeeding two years records taken at intervals have shown the picture now known to be characteristic of disturbances which follow lesions of the caudate and putamen.

In all records from animals with such ablations these characteristic changes have appeared although the degree of abnormality varies, possibly with size of the lesion: (i) A marked diminution in the amount of fast 15–20

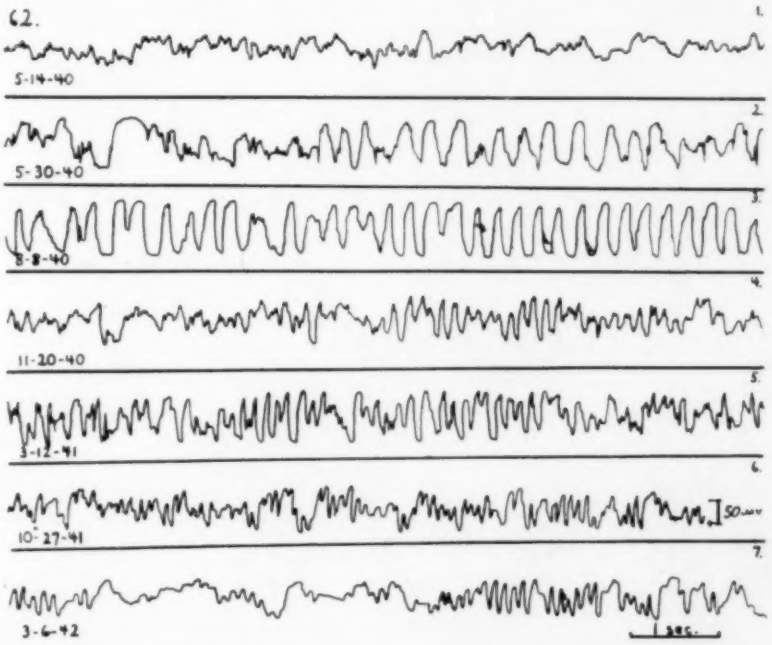

FIG. 4. Effects on EEG of ablation of head of left caudate nucleus on May 20, 1940 and of head of right caudate nucleus on August 16, 1940. This infant was born April 27, 1940. Hypersynchrony is invariably present. Note increase in rate characteristic of a normally developing animal during first two years of life.

per sec. rhythm which at times disappears altogether. (ii) An increase in the regularity of the pattern and a diminution in total variability. (iii) The most noticeable change is in the medium 5–10 per sec. rhythm which becomes accentuated and forms the "saw-toothing" described by Case and Bucy (1) or "hypersynchrony" as designated by Jasper (11, 13), (Fig. 3, 4, 5).

Such hypersynchrony may appear only in short bursts between periods of more or less normal record, or may continue for many minutes. It is sometimes entirely absent, and then may appear the next day in the record of the same animal. When present, it usually is seen in all leads, but at such times the amplitude is greatest in the frontal regions (1E, 4E) and in the interhemisphere records (1–3 and 4–6). In all leads, amplitude is increased during these periods of hypersynchrony. Although such changes have per-

sisted for as long as two years, the longest period for which animals have been kept following such operations, they tend to be more marked in the weeks immediately after operation and become less extreme with time. These animals with lesions restricted purely to the basal ganglia have no clinical signs of neurological deficit of any kind.

D. Effect of combined lesions of cortex and basal ganglia (15 monkeys). These combined lesions produced the most definite and extreme changes in EEG which were noted. The cortical lesions were extirpations of either areas 4 or 6 or both. They were made in conjunction with ablations from

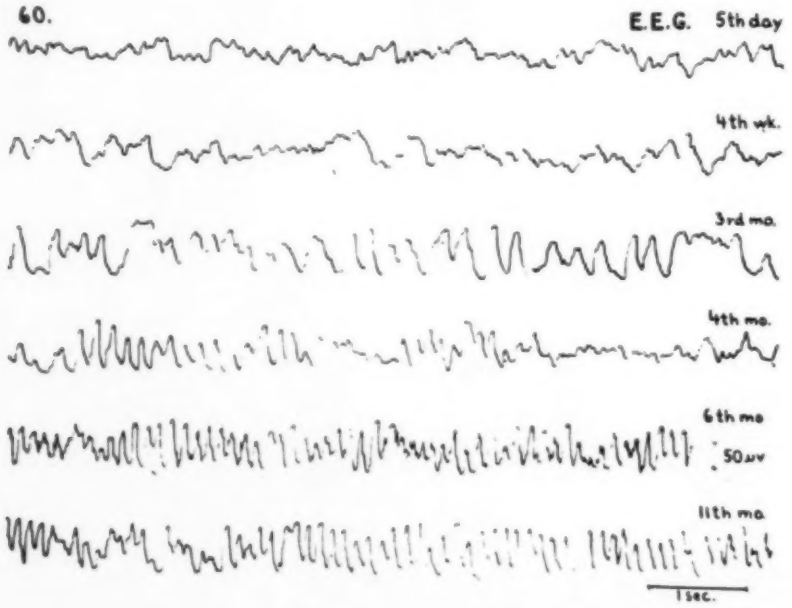

FIG. 5. Hypersynchrony appearing in an infant *Macaca mulatta* which had seriatum ablation of areas 4 and 6 and caudate nuclei; from left side during 5th week and from right during 5th month of life. Note increase of rate with age.

caudate or putamen or both. In every instance the change appeared immediately after operation, (many of the records were taken 24 or 48 hours after the lesion had been made) and persisted for as long as the animals lived (2 years in some cases). They were often more extreme immediately following operation and became less pronounced with time. They were always more pronounced in those animals operated in infancy (6 monkeys) than in those operated at a later date (9 monkeys). Epilepsy when present, occurred only during the first weeks following operation and later disappeared.

Figures 5, 6 and 7 show the type of alterations in EEG which are characteristic of these combined lesions. They are, in general, similar to those described by Jasper in his appraisal of the cortical potentials of epileptic humans (11).

Hypersynchrony appeared as the most pronounced change. It was extremely marked, being present at times only in short bursts, but running usually throughout the records and being present in all leads. The amplitude of the leads to ear (1E, 2E, etc.) was always very great, that of the potentials

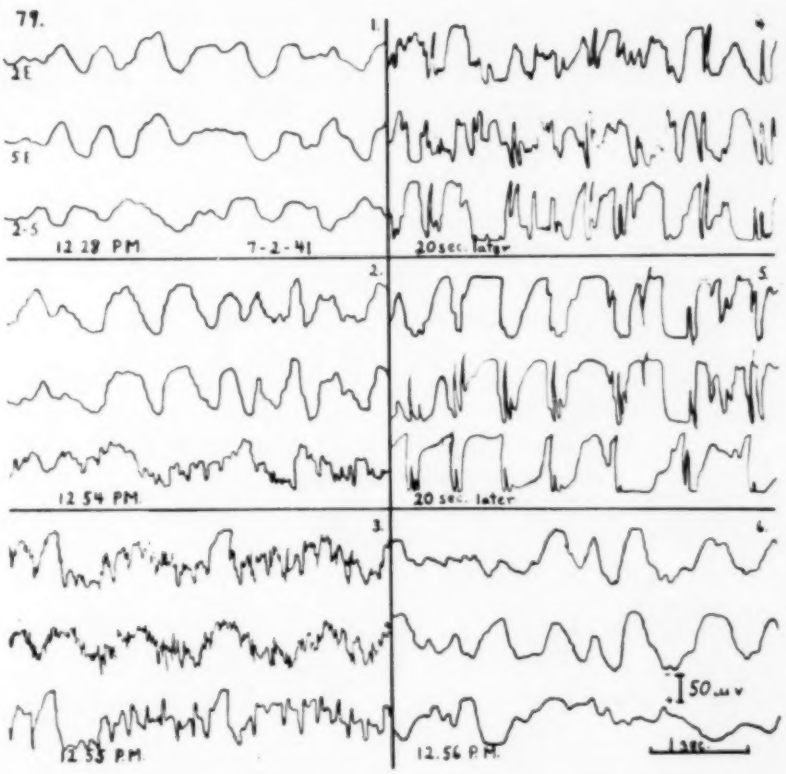

FIG. 6. Episodes from an attack, of tonic clonic generalized epilepsy in a *Macaca mulatta* 48 hours after bilateral ablation of area 6 and caudate nucleus.

between the hemispheres (1–4, 2–5, 3–6) decreased. The hypersynchrony, then, did not appear definite in the interhemisphere leads of low potential, while it was extreme in the intra-hemisphere recordings. The fast potentials during these episodes of hypersynchrony are invisible, and at all other times are diminished. There are no slow potentials, so that the appearance of the record is characteristic.

Records of epileptic attacks have been made in five of these animals, three of them infants, one a young animal and one a nearly mature female. Four of these five had clinical grand mal attacks at the time when the records were being taken. The fifth, the older animal, has never been observed in an epileptic attack, although its other clinical symptoms and the nature of its lesion are the same as those of the others. In each animal, attacks appeared on the day after operation and have continued for some days or

weeks. In each, epilepsy was present at times, but absent at others. Thus, daily records would show, on two or three successive days, either grand mal or the "spiking" records which indicate cerebral hyper-excitability. There might then be a day or several days without epileptiform potentials and then a seizure would be found at a later date. In each animal the epilepsy disappeared by the end of the first postoperative month but the hypersynchrony

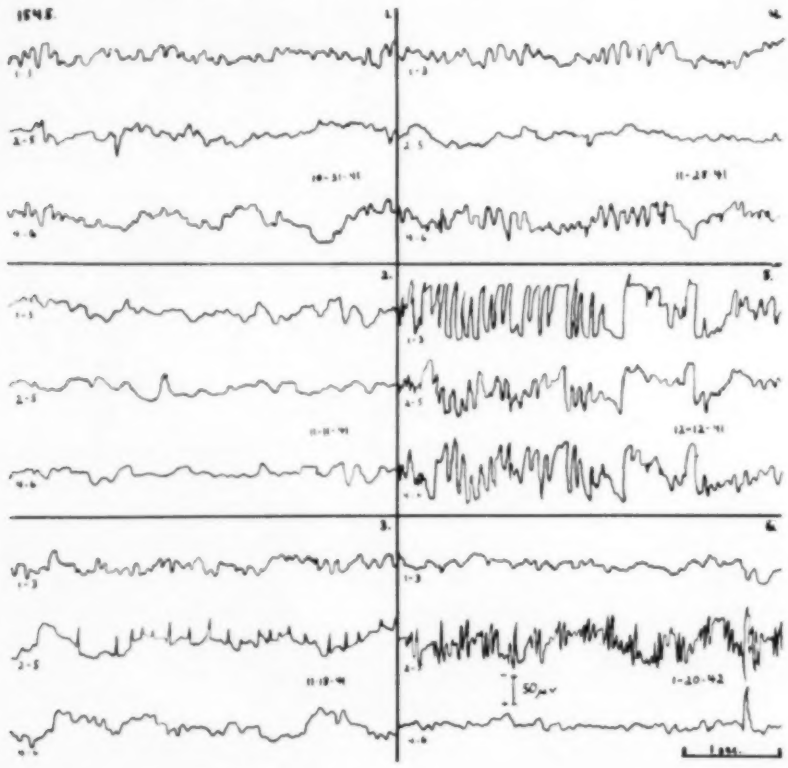

FIG. 7. Changes in EEG following bilateral ablation of area 6 and caudate nucleus on Nov. 10, 1941 in a *Macaca mulatta* (weight—4.0 kg.) 1, Normal; 2, Postoperative slowing; 3, Focal irritation in leads 2–5; 4, Hypersynchrony; 5, Epileptic attack; 6, Focal irritation persisting 6 weeks later.

remained. All these animals had, in addition to epilepsy, marked motor deficit; tremor, spasticity and impairment of skilled voluntary movement.

Figure 6 gives excerpts from a record taken throughout an attack of generalized epilepsy. During this period the animal showed first clonic and then tonic generalized convulsions. The entire attack lasted about six minutes. It was one of many witnessed during the first three days subsequent to simultaneous bilateral removal of areas 6 and head of caudate nucleus from a 4-week-old infant.

Figure 7 shows the records of several days taken after a similar ablation. This is the animal which was never seen to have clinical epilepsy, yet

throughout a period of more than three weeks, had records indicating various types of increased excitability of cortex. In the illustration excerpts have been chosen showing isolated spiking of one lead only, spiking of all leads, and parts of a generalized attack. This animal is now four months postoperative, and continues to show hypersynchrony, but without epilepsy. In this monkey (Fig. 7) the focus of excitability could be at times isolated to one area of cortex. At other times it spread to involve all other areas as well. Thus, by changing combinations of the leads, it could be seen that the area of largest and most constant spiking was in the region of lead 2, or just above the ablation on the left side.

Discussion

A comparison of the pre- and postoperative EEGs of 41 monkeys with lesions in cortex and basal ganglia has brought out some consistent and at times surprising points of interest. The most striking of these is that there is little or no change in the pattern of cortical potentials if the cerebral cortex alone is damaged, while lesions of the basal ganglia together with cortex, produce profound and permanent rearrangement of this pattern.

Although it is commonly thought that the electroencephalogram is a picture of the sum of *cortical* potentials alone, it has been shown not only by the present series of experiments but by much previous work, that activity of cortex and basal ganglia are intimately related. It is an old and consistent finding that stimulation of the cerebral cortex and of the cortex alone can produce epilepsy, and that no stimulation of basal ganglia alone results in any type of motor response (22). Yet combined stimulation (19) or ablation (15, 16) of basal ganglia and motor areas of cortex will materially alter the pattern of the motor response which is integrated through the cortex. Furthermore, it is extremely difficult to produce experimentally any type of permanent or recurrent epileptic state by injury to cerebral cortex alone although, recently this has been successfully done as reported by Pacella, Barrera and Kopeloff (20). To this can be added the well known clinical finding that epilepsy develops frequently only long after the cortical injury has been incurred. Foerster and Penfield (10) have shown that such epilepsy which first appeared in a large series of cases of brain injury many years after the initial trauma is due to scar formation and retraction distorting the ventricles as shown by pneumoencephalogram. Such distortion would very probably alter conditions in the basal ganglia since the caudate nuclei form the lateral boundaries of the lateral ventricles. That excision of cortical scars (21) abolishes epilepsy in these cases must be explained by the fact that the excitable foci for the attack lie in cortex, and that no epilepsy is possible without active cortical cells lying somewhere in the reverberating circuit of electrical activity.

That the cortex and in particular the motor areas of the frontal lobe, areas 4 and 6 of Brodman and the strip area 4-s (9) lying between them, are intimately concerned in integration of "voluntary" motor acts has been shown in various ways. Combined ablation of these areas with caudate pro-

duces in the monkey or chimpanzee a type of motor disorder which is characteristic for this combined ablation (16). To this should be added the information obtained by Dusser de Barenne and McCulloch (3, 4) that there are reverberating circuits defined by strychninization of various areas of cortex which pass from cortex directly to caudate and putamen and thence back to cortex either directly or via thalamus. Interruption of these pathways either by excision of area 6 and caudate nucleus or by large bilateral excision of caudate and putamen alone will be followed, in the monkey, by the appearance of dyscoordinations and dysrhythmias which are another indication of disturbance in rhythmic pattern.

There is some clinical evidence that the basal ganglia affect the EEG, for Gibbs and Gibbs (7) have seen changes in records from patients with athetosis and chorea although those with Parkinson's syndrome did not show similar abnormalities. Dynes and Finley (2) studying narcolepsy report characteristic patterns which are altered by the benzedrine drugs.

In the light of previous experience with man, it is surprising that there are no focal signs in the EEG of the monkey following specific lesions. Moreover, when changes develop following combined lesions of cortex and basal ganglia they were, with few exceptions, manifest in all leads. Only when true epilepsy was present or in the preepileptic state of the same animals did occasional isolated foci of unusual excitability occur.

The non-specific reaction of the potentials to trauma at any point of the central nervous system thus far injured may be compared to the changes found in man after injury to the head (12, 18). There is the same slowing of rate and smoothing out of amplitude and pattern. The degree and rate of recovery is related in the same way to the extent of the lesion. That concussion is not the same as experimental ablation probably accounts for the fact that the picture in man is complicated in some cases by the appearance of epileptiform patterns (12) although this does not appear in the experimental monkey.

There are data from several aspects of this investigation which show that *after* and not *during* the time of cortical excision, changes take place which alter cortical potentials. First, no alteration in pattern was produced in acute preparations immediately following excisions of any portion of the nervous system although removal of some of these same areas was followed by profound changes in the EEG of the chronic preparations. Two factors might effect this difference. Either there must be some rearrangement of remaining tissue which accounts for the eventual changes, or else, the anesthesia (dial in these cases), may alter the acute picture in some way so that immediate changes are not apparent. The matter could be easily further investigated, and must be before any definite conclusion can be reached.

Other signs of rearrangement after operation appear in relation to the non-specific changes which follow cerebral operation. As described above, these changes are sometimes not apparent on the first day after operation but only on the second day. In this instance they may be correlated with the reaction of tissue to injury.

The most important finding brought out by these investigations has been the production of epilepsy both clinically and in the EEG and its relation to the basal ganglia. All the changes in pattern, hypersynchrony and irregularities, as well as true epileptic seizures can, according to the descriptions given by Jasper (11, 13) and Gibbs and Gibbs (7), be attributed to the epileptic state either between attacks or during an acute episode.

The conditions essential to cause true epilepsy in these animals are not clear, for in only five out of fifteen instances in which bilateral ablation of area 6 and the caudate nucleus has been performed did these attacks appear. However, in every case the affected animal has been one which has showed the most severe clinical symptoms of tremor and spasticity. It is likely therefore that size of lesion may determine this epileptic factor. In contrast those monkeys with pure caudate and putamen lesions, which had minimal clinical symptoms all developed hypersynchrony but no epilepsy.

The fact that monkeys operated upon in infancy have uniformly exhibited more striking changes than have those operated upon at a later age is consistent with what is known of similar conditions in man, particularly if these changes in the monkey are related to epilepsy. For it is well known that infants are particularly susceptible to epilepsy under almost any condition that can bring on seizures whether fever, non-specific trauma or focal cerebral injury. In all infants there must then be some condition of the cerebral cortex or basal ganglia which permits synchronization of cortical potentials into patterns characteristic of epilepsy more readily than in the adult. The greater tendency of the young of man to other disorders of the basal ganglia such as chorea and athetosis is also known as is the greater capacity for reorganization of the cerebral cortex for physiological activity (14).

Conclusions

Lesions have been made in cerebral cortex and basal ganglia of 41 monkeys, and electroencephalograms (EEG) recorded before and after operations.

1. In every instance there is a temporary change in EEG which appears during the first or second postoperative day, and which consists of flattening and slowing of the waves of medium frequency. It is transient; it is independent of the specific area injured; but it is more pronounced following larger lesions.

2. In acute experiments under dial anesthesia, lesions of cortex or basal ganglia produce no change in EEG.

3. Following lesions restricted to cerebral cortex there is no significant change in EEG except the transient one described above.

4. There was no focal effect of lesions of the cortex, and usually none following combined lesions of cortex and basal ganglia. In a few instances focal epilepsy could be seen.

5. Lesions of head of caudate nucleus or putamen, or of both, were followed by marked changes in the pattern of cortical potentials. Hypersyn-

chrony of the 8–10 per sec. waves became intensified, and the 15–20 per sec. waves became less marked or vanished.

6. Combined lesions of the motor areas of the cortex with the basal ganglia caused the most marked changes. Hypersynchrony appeared and persisted for as long as two years. True epilepsy, both clinical and in EEG were found in 5 out of 15 animals.

7. All the lesions which caused changes in EEG produced the most extreme changes in those animals which had been operated upon in infancy.

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MODIFICATION OF CORTICAL ACTIVITY BY MEANS OF INTERMITTENT PHOTIC STIMULATION IN THE MONKEY

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IT HAS BEEN SHOWN by Bartley and by Bishop that the cortical activity (electrical) of the rabbit may be markedly altered in amplitude and rate by intermittent photic stimulation of the retina. Cortical responses approach their maximal amplitude when the flashes of light occur at the frequency of the alpha rhythm (2, 6, 7). However, the alpha frequency may itself be raised or lowered in the rabbit under certain conditions by corresponding rates of stimulation (5). In the cat, Bishop and O'Leary have found that while the cortical activity may be enhanced in amplitude by electrical stimulation of the optic nerve at the alpha frequency, the results for photic stimulation were less distinct (9, 8). In man, Adrian and Matthews (1) and others have found, as in the case of rabbit, that the alpha rhythm may be substantially raised or lowered in rate by corresponding rates of photic stimulation. The present investigation affords evidence that in the monkey the cortical activity may be markedly altered in rate and amplitude by intermittent light, and that with the same luminous flux, maximal cortical responses are obtained at particular frequencies of stimulation.

METHOD

Experiments were carried out on 12 adult monkeys (*Macaca mulatta*). Electroencephalograms were simultaneously recorded on paper tape from both occipital regions by means of the three-channel amplifying equipment† and procedures (adapted for monkey) described by Case and Bucy (11). Flash intervals were also recorded continuously on the tape by means of a photocell pickup working into the third amplifying channel.

Stimulation was carried out with the monkey in a darkened, electrically shielded cage. The animal was attached to an animal board and placed before an aperture in one side of the shielded cage in such a manner that flashes of light introduced through the aperture gave an equal illumination to both eyes of 120 footcandles. Light from a tungsten source was interrupted at various frequencies by means of an interposed episcotister driven by a DC motor. Light-dark ratio was 1/1.

Quantitative analysis was made of all records. At intervals of one foot of tape, each record was sampled for 10 successive waves until 25 such groups were obtained for each animal (tape speed of 3 cm. per sec.). Each set of 250 waves was then measured for amplitude in units of 0.25 mm. under a reading lens giving 2.5 times magnification. The measurements were then separated into two groups depending upon whether or not the cortical frequency corresponded with the flash frequency. For convenience of comparision, the total amplitude per second was calculated for each frequency by multiplying the average amplitude by the frequency. The values for cortical frequencies which did and did not correspond to flash frequency are the basis for the curves shown in Fig. 1, 2 and 3. Ac-

* The animals employed in this investigation were purchased by funds granted to one of the authors (Walker) by the Ella Sachs Plotz Foundation.

† The authors are indebted to Dr. Theodore J. Case for the use of this equipment.

curacy of amplitude measurements was checked by re-measuring 200 waves from a tape selected at random and was found to be better than 96 per cent in terms of the average deviations. Both ascending and descending series of flash frequencies were presented in each experiment to reduce the probability of chance correspondence between cortical frequency and flash frequency.

RESULTS

Driving effect of intermittent light. Substantial evidence of an effect of intermittent photic stimulation upon the cortical activity was found in the record of every monkey. This effect was reflected over a range of several cycles in two ways: (i) by increased amplitude per unit of time of the cortical activity, and (ii) by regularization or increased stability of the cortical rhythm. Over the range in which driving was produced, the effect was present on an average of 55 per cent of the time for the group as a whole and ranged from 28 to 78 per cent for individual monkeys. Representative curves indicating the magnitude of the driving effect are shown in Fig. 1, 2, and 3.

Range of driving effect. The range of frequencies at which driving occurred varied in different animals from 7.5 cycles to 2.0 cycles per sec. and averaged 5.0 cycles per sec. for the group as a whole.

Relation of amplitude per unit of time to flash frequency. Examination of the amplitude maxima obtained during driving (*i.e.*, with cortical frequency synchronous with flash frequency) revealed that they tended to cluster in a limited range. A plot of the maxima against frequency is shown in Fig. 4. It may be noted that for the twelve monkeys (plus a repeated record made on one animal) 9 amplitude maxima fall within a frequency range of one cycle per sec., *i.e.*, within the range from 10.5 to 11.5 flashes per sec. This fact becomes of particular interest when contrasted with the non-driving condition. Here the amplitude maxima were found to be distributed over a much wider range (7 to 29 cycles per sec.) with no suggestion of clustering. In no instance did more than two animals have amplitude maxima falling within one cycle of the same frequency.

Regularization of rhythm. Regularization or stabilization of rhythm was a marked feature of the records obtained under conditions of driving as contrasted with non-driving. This is illustrated in the sample records reproduced in Fig. 5.

Production of multiple frequencies. Further evidence of the effect of the intermittent flashes was the not uncommon appearance of cortical frequencies exactly double or triple that of a given flash frequency. A similar result has been obtained by other investigators (cf. 1, 2, 8).

Auditory component ineffective. Control records obtained with the apparatus in operation but without the light flashes revealed no evidence of driving.

Effectiveness of fluorescent light in driving cortical activity. Preliminary investigations were made of the effectiveness of a G.E. 15 watt daylight fluorescent lamp, made intermittent at various frequencies by means of a multivibrator circuit, upon driving of cortical activity. Substantial driving was obtained in nine monkeys with flashes of 44 footcandles in brightness with a light-dark ratio 1/1. In twelve monkeys, no driving was produced by fluorescent light under the conditions otherwise identical with those described

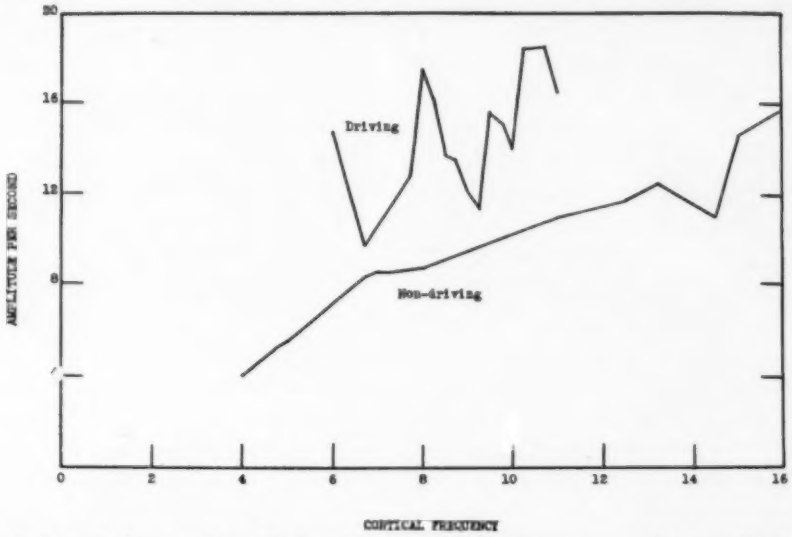

FIG. 1. Graph for monkey X showing the amplitude per second of cortical activity during intermittent photic stimulation (upper curve) when cortical frequency corresponds with flash frequency and (lower curve) when it does not correspond with flash frequency. The amplitude per second was calculated by multiplying the average amplitude of 10 successive cortical w s by their frequency (cf. text).

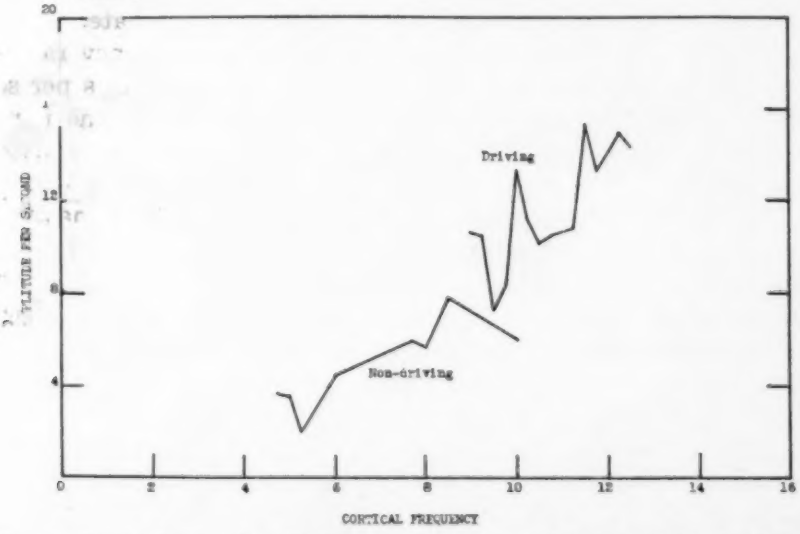

FIG. 2. Graph for monkey Y similar to Fig. 1 but showing relatively discrete driving and non-driving spectra of cortical frequencies.

above for the tungsten source of light. In comparison then with the tungsten source, with which driving was produced in all twelve monkeys, fluorescent light was effective in only 43 per cent of a second group of 21 monkeys. The possible role of such factors as flash intensity, light-dark ratio and spectral composition in producing this difference is considered in a separate publication.

Discussion

Little is known concerning spontaneous frequencies which occur in the electroencephalogram of the monkey. For *Macaca mulatta* frequencies of 7 to 8 per sec. have been reported by Kennard and Nims (13) as characteristic of older animals, in contrast with frequencies of 4 to 5 per sec. seen in the records of animals 6 to 8 months in age. Greater variations were found in the records of the older animals. It is of interest that amplitude maxima under driving conditions were found by us to cluster between 10.5 and 11.5 cycles per sec. Only three of our animals produced amplitude maxima in the region of 6 to 8 per sec. and in one animal maximum amplitude was reached at 13.7 cycles per sec. (cf. Fig. 4). Under non-driving conditions it was found that waves of large amplitude occur at frequencies as high as 29 per sec. (cf. Fig. 3). No driving was found above 13.7 per sec. and only two monkeys drove above 12 per sec. There was in fact a marked tendency noted in the records for the upper limit of driving and the amplitude maxima to fall at the same frequency. No explanation for this abrupt cutoff in driving can be offered at this time.

The sensory resultant for man of frequencies of intermittent light below fusion is flicker. Two regions in the range of flicker are known to produce *enhancement* of apparent brightness. The first of these was reported by Brücke (10) in 1864 to be in the region of 17 to 18 cycles per sec. He observed that the white areas of a rotating disc made up of black and white sectors became more brilliant at this rate of rotation than when stationary. The second range of frequencies known to produce brightness enhancement was reported by Bartley (3) in 1938. Using transmitted light, Bartley measured the average brightness level for successively lower flash rates. He found that the brightness of the "average" level begins to rise until it reaches, then exceeds a level equivalent to that obtained with continuous illumination. Maximum enhancement is reached at 8 to 10 flashes per sec. At this point the apparent brightness is the reciprocal of the fused brightness of the intermittent field. Bartley's findings on brightness enhancement were recently confirmed by Halstead (12) who eliminated the possible origin of the effect in the pupillary and accommodative reflexes by studying these relations in a normal subject with these reflexes eliminated by scopolamine.

It seems probable that the Brücke effect and the Bartley effect are distinct phenomena in the range of flicker. The Brücke effect is obtained by judging the bright phase of the fluctuating component of the intermittent source. The Bartley effect is obtained by judging the steady component of an intermittent test-field but does not appear when the fluctuating field has an illuminated surround (3, 4). The Brücke effect was obtained under conditions in which the fluctuating field had an illuminated surround.

It is not known whether either or both of these effects are operative as mechanisms in the brightness vision of the monkey. Bartley has interpreted the correspondence between the region of maximal brightness enhancement and the alpha frequency of 8 to 10 per sec. in man and the fact that optimal

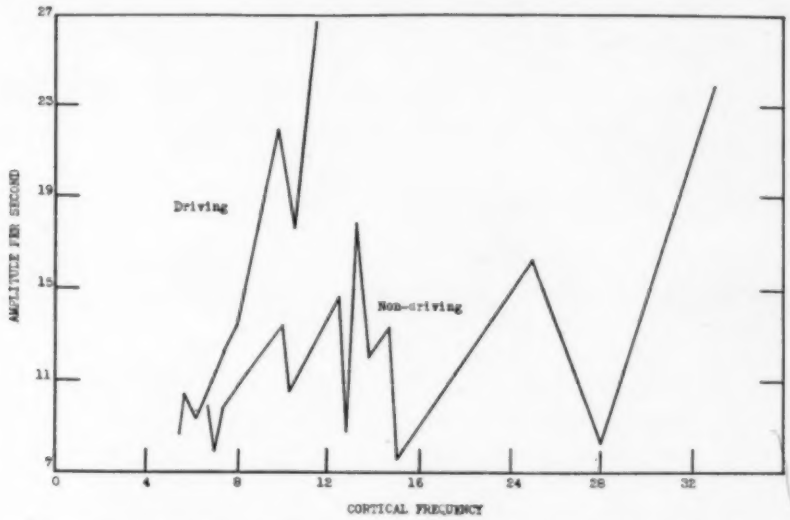

FIG. 3. Graph for monkey W similar to Fig. 1 but showing a narrow driving spectrum and a broad non-driving spectrum of cortical frequencies.

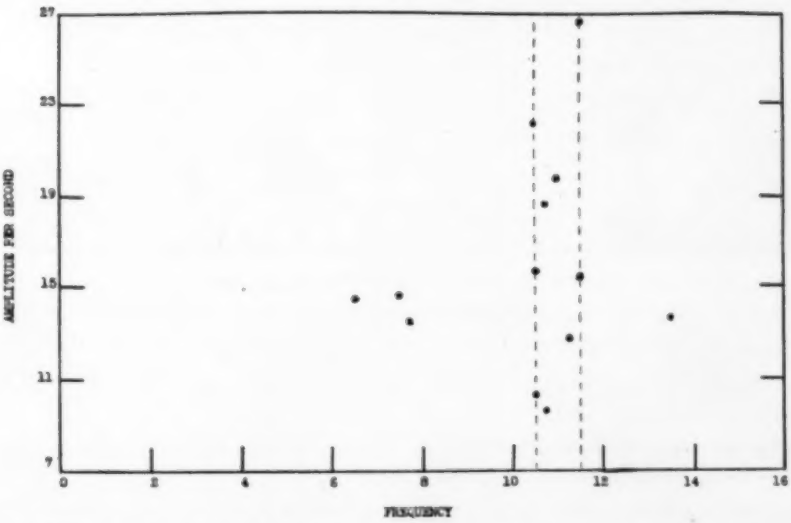

FIG. 4. Graph showing the clustering of the amplitude maxima, obtained under conditions of driving in twelve monkeys (with repeat record on one animal), at a frequency of approximately 11 per sec. No clustering was found under conditions of non-driving (cf. text).

driving occurs at the alpha frequency as indicating the operation of a common mechanism in the brain rather than at the periphery (5). If this proves to be sound, then it seems probable from the results obtained in our experiments that the region of maximal enhancement of brightness of intermittent light for monkey will be found in the neighborhood of 11 flashes per sec., since maximal alteration of the cortical activity occurs in this region.

FIG. 5. Representative electroencephalographic records showing:
A. Driving at a frequency of approximately 11.5 per sec. in the left occipital lead only.
B. Non-driving bilaterally at a flash frequency of 9 per sec.
C. Driving in both occipital leads at a frequency of 6 per sec.
(In each record 1—left occipital lead, 2—right occipital lead, L—light flashes as recorded from a photoelectric cell. The time in seconds is indicated at the bottom of the figure. The units at the right of the tracings indicate a deflection of 300 μV.)

SUMMARY

Evidence was found of an effect of intermittent photic stimulation upon the cortical activity of the monkey (*Macaca mulatta*).

The effect was reflected by increased amplitude of cortical response and by stabilization of cortical rhythm. The effect is similar to the driving effect reported by Bishop and his co-workers for rabbit and cat and by various investigators for man.

The range of frequencies found to produce maximal cortical responses in monkey was from 10.5 to 11.5 per sec.

The driving effect was found to be present in 100 per cent of twelve monkeys.

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RELATION OF FIBRILLATION TO ACETYLCHOLINE AND POTASSIUM SENSITIVITY IN DENERVATED SKELETAL MUSCLE

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WHEN the motor nerve to a skeletal muscle is cut the muscle involved undergoes several distinctive changes. At about the time the nerve degeneration is complete fibrillation commences. This is accompanied by characteristic action potentials (1, 3, 6, 7). Concurrently the muscle also develops an increased sensitivity to acetylcholine. Close arterial injection of a large dose of this substance (1) into a normal muscle produces a brisk contraction. Distant injections are without effect. With denervated muscle, however, administration of a minute dose of acetylcholine, by either the intra-arterial or intravenous route, produces a characteristic response (1). This consists of an initial "quick" movement of the muscle, accompanied by an outburst of action potentials, and is followed by a prolonged contracture. A similar difference in the nature of the contraction in normal and denervated muscles was observed following the arterial injection of potassium chloride but no difference in sensitivity was reported (2).

Quinine depresses, but does not completely abolish, the response to acetylcholine administration in cats (4). Oester and Maaske (5), working with dogs, found that large doses of quinidine almost completely abolished the effect of intra-arterially injected acetylcholine. In neither of these investigations, however, was this acetylcholine antagonism considered in relation to the effect of quinidine on fibrillation. In previous investigations (6, 7) fibrillation was abolished in the denervated muscle of the rat with adequate dosage of quinidine. The experiments to be reported here were undertaken with the object of comparing this property of quinidine with its action on the sensitivity of these muscles to acetylcholine and potassium in the hope of effecting a further elucidation of the genesis of fibrillation in denervated skeletal muscle.

METHODS

Albino rats, weighing 200 to 350 gm., and on an adequate diet, were used throughout. The gastrocnemius-soleus muscle group on one side was denervated, under ether anaesthesia, by removing a length (0.5 cm.) of the sciatic nerve. The animals were tested between the 6th and 14th days after denervation.

Under light ether anaesthesia, a skin flap was cut, and the tendon of the denervated muscle group dissected out and detached from its bony insertion. The lower limb on that side was then immobilized with metal clamps running from rigid uprights to the femur and foot. A cord from a small hook through the muscle tendon was attached to an isotonic lever writing on a smoked drum. The action potentials were led from the muscle belly through

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two fine needles and amplified. They were recorded photographically and rendered audible through a loud speaker. With the abdomen open, the iliac artery supplying the opposite limb was clamped off. All injections were then made into the lower aorta in constant volumes (0.25 cc.) and at a fairly constant rate. The drugs were in aqueous solutions. Acetylcholine bromide was used in various dilutions giving a dosage range from 0.002 γ to 2 mg. Potassium chloride was used in solutions containing 0.2, 2.0, and 20 mg. per cc. The quinidine solution contained 25 mg. quinidine sulphate per cc. The mechanical and electrical responses of the muscle to acetylcholine or potassium were noted before and after quinidine administration.

Results

1. *Effect of quinidine on fibrillation and acetylcholine sensitivity.* This series was made up of 29 animals. In all of them the characteristic action po-

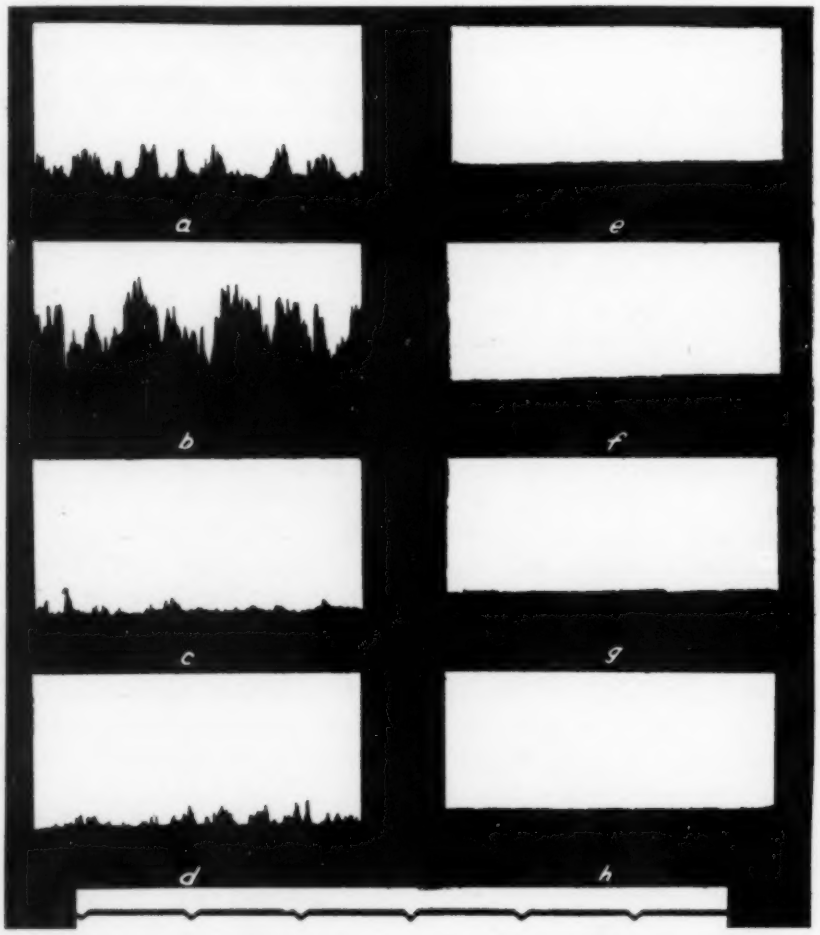

FIG. 1. Action potentials from gastrocnemius-soleus muscle of rat 8 days after denervation.

(a) Fibrillation; (b) intra-arterial injection of 0.02γ acetylcholine; (c), (d), 2 and 5 sec. after (b).

(e), (f), (g), (h) Similar records after 6 mg. quinidine sulphate given intra-arterially.

Time; 1 division = 0.2 sec.

tentials of fibrillation (Fig. 1, a) were readily detected by ear and photographed (6). Intra-arterial injections of acetylcholine produced results similar to those of Brown (1). There was first a rapid contraction accompanied by an electrical outburst ("quick phase"). This was followed by a prolonged slower mechanical movement during which all action potentials, including

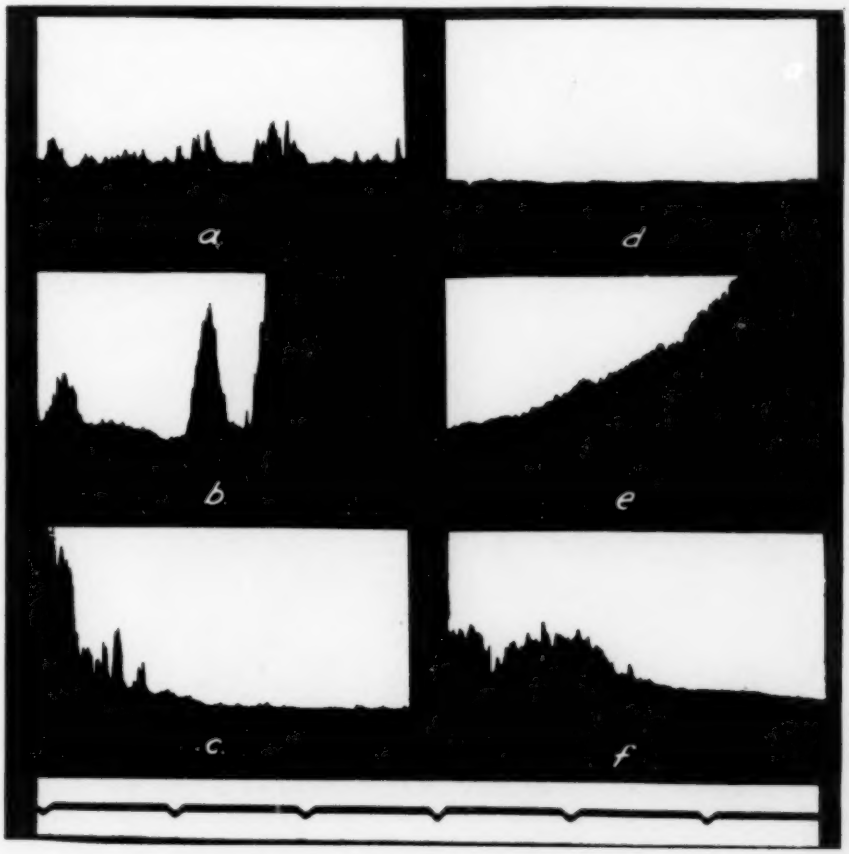

FIG. 2. Action potentials from gastrocnemius-soleus muscle of rat 8 days after denervation. (a) Fibrillation; (b) intra-arterial injection of 2.0γ acetylcholine; (c) 2 sec. after (b). (d), (e), (f) Similar records after 6 mg. quinidine sulphate given intra-arterially. Time; 1 division = 0.2 sec.

those of spontaneous fibrillation, were abolished ("contracture phase"), or were too slow to be recorded by our apparatus. The duration of this second phase varied with the acetylcholine dosage and, with the smallest doses (0.02–0.002γ), was transient or absent (Fig. 1, a, b, c, d). With larger doses (2.0γ–2 mg.), the electrical outburst with the large initial contraction blocked the amplifier temporarily (Fig. 2, a, b, c) before the prolonged second phase appeared. The first three tracings of Fig. 3 are representative of mechanical records from such experiments. The two-phase response with

2.0γ administration is evident. Such division is not seen, however, with the lower dosages. In this experiment, as in 21 out of the group, 0.002γ acetylcholine was the smallest effective dose. In the remaining 8 experiments, 0.02γ was necessary to evoke a response. In contrast, the smallest effective dose for normal muscle, was 2 mg.

Quinidine, in 6 mg. doses, produced an immediate cessation of fibrillation in all cases. The action potentials were abolished, with no evident mechani-

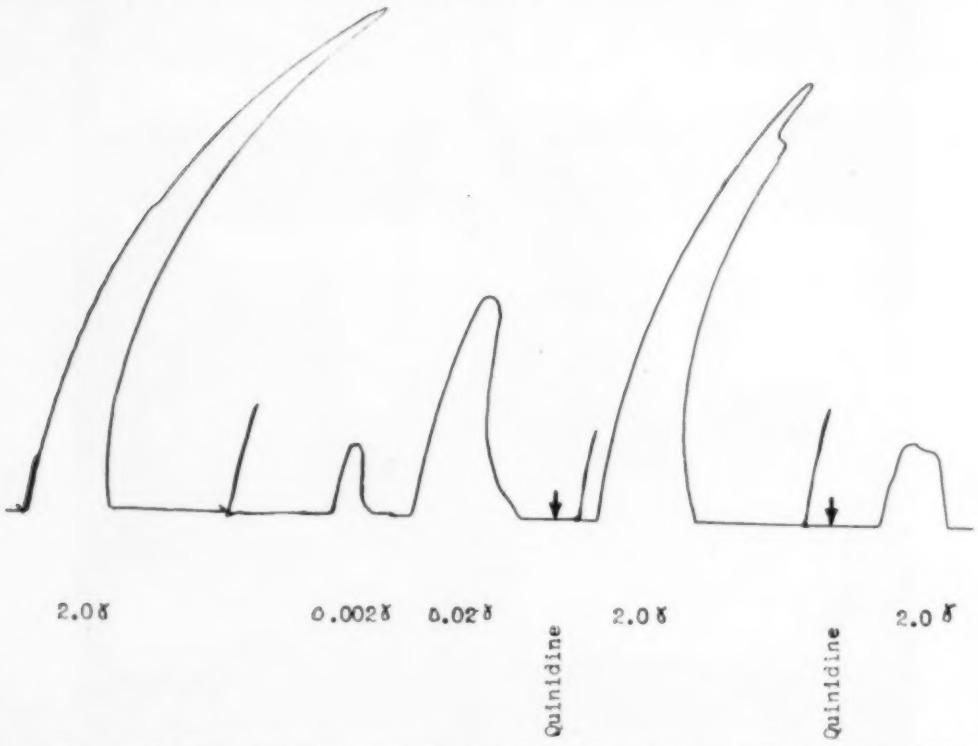

FIG. 3. Mechanical responses of denervated muscle to intra-arterial injections of 2.0, 0.002, 0.02, 2.0, 2.0γ (from left to right) of acetylcholine. At arrows, 6 mg. quinidine sulphate given intra-arterially 1 minute apart.

cal movement of the muscles. The duration of this arrest was not followed beyond a period of 15 minutes but it must, in most cases, have been considerably longer. During such periods of absent fibrillation, the effects of acetylcholine administration varied with the dosage employed. Small doses which normally produced definite contractions, were completely ineffective, mechanically and electrically. The flat baseline after quinidine (Fig. 1, e, f, g, h) was unaltered by the injection of 0.02γ acetylcholine. This dose, previously administered (Fig. 1, a, b, c, d), had produced a definite outburst of action potentials. The responses to higher concentrations of acetylcholine were opposed to a less degree. In Fig. 2 (d, e, f), although fibrillation had been abolished by quinidine, 2.0γ acetylcholine still produced considerable electrical

effect. The mechanical counterpart is seen in Fig. 3, where, after quinidine, only partial reduction of contraction height occurred. With larger doses of acetylcholine (up to 2 mg.) there was only slight reduction in the outburst of action potentials, and no differences in the mechanical records were seen.

2. *Effect of quinidine on fibrillation and potassium sensitivity.* The relative effects of quinidine and potassium were studied in a similar fashion in 12 experiments. Here, the results were less clear-cut than was the case with acetylcholine though the general trend was the same. In the first place, though present, there was much less difference between the sensitivities of normal and denervated muscles to potassium administration. This is seen clearly in Table 1 where the minimal effective doses are compared for the

Table 1. Minimal effective dosage of potassium chloride producing mechanical movement in denervated and normal muscles.

	KCl dosage		
	20 mg.	2.0 mg.	0.2 mg.
Denervated muscle	1 experiment	4 experiments	7 experiments
Normal muscle	5	5	0

two types of preparation. Although one denervated muscle required 20 mg. of potassium chloride to elicit a response, yet in most cases 0.2 mg. produced a contraction. On the other hand, 5 normal muscles were effectively stimulated by 2.0 mg. of potassium chloride, 5 required 20 mg., and none showed a response to the smaller dosages.

After effective quinidine arrest of fibrillation, the sensitivity of denervated muscle to potassium administration was reduced in a fashion similar to that seen with acetylcholine. Doses of 2 mg. or less were ineffective. With higher concentrations of potassium chloride, however, considerable variation was seen. The response to injections of 20 mg. was unaffected by the quinidine in 3 cases. In 4 cases, some reduction was seen and in 5 instances the potassium was completely ineffective.

Discussion

In previous papers (6, 7) it has been shown that the fibrillation resulting from denervation of skeletal muscle can be abolished by adequate administration of quinidine. When this substance is injected intraperitoneally there is a diminution, in many cases a complete cessation, of fibrillation which lasts up to 9 hours. Denervated muscles are much more sensitive to acetylcholine than are normal muscles (1). On this basis it has been suggested (3) that fibrillation is the result of an asynchronous response to the acetylcholine normally present in tissue fluids.

The action potentials of fibrillation are of a degree comparable to those induced by the intra-arterial injection of relatively small doses of acetylcho-

line (Fig. 1, a, b, c, d) or of potassium chloride. They differ greatly from the electrical outbursts produced by large doses. Intra-arterial injections of quinidine, while having less effect on these large outbursts, completely abolish the responses to small doses of these substances (Fig. 1, e, f, g, h). Apparently quinidine, in concentrations which abolish fibrillation, renders denervated muscle unresponsive to doses of these other agents which ordinarily produce a comparable degree of excitation. These experiments, then, support the thesis that fibrillation arises from an increased sensitivity of the muscle to chemically-induced excitation and indicate that acetylcholine, potassium or both may be the cause of fibrillation. They do not, however, shed light on the possible relative rôles of acetylcholine and potassium in the production of the fibrillation of motor denervation.

Summary

Denervation renders skeletal muscle much more sensitive to acetylcholine and, less markedly, to potassium chloride. Small concentrations of these agents, when injected intra-arterially, produce action potentials comparable to and superimposed on those of fibrillation. This excitation is abolished by quinidine in doses which will arrest fibrillation. It is suggested, therefore, that the fibrillation seen in skeletal muscle after lower motor neurone denervation arises from an increased sensitivity of denervated muscle to chemically-induced excitation. The present experiments indicate that acetylcholine, potassium or both may be the usual causative agent or agents.

We are grateful for the interest taken in this work by Professor C. H. Best.

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CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF PROPRIOCEPTORS AND EXTEROCEPTORS IN THE RAT*

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INTRODUCTION

THE AFFERENT TRACTS from the spinal cord to the cerebellum have been generally considered to carry proprioceptive sensory impulses exclusively. Every modern textbook of neuroanatomy assigns the spino-cerebellar tracts to the proprioceptive system. The tendency to link the cerebellum exclusively with proprioceptive sensation has been so marked that the term muscle-sense has been used by many interchangeably with spino-cerebellar when speaking of the impulses and connections from the spinal cord to the cerebellum. Although cerebellar deficiencies show themselves both clinically and in experimental animals as abnormalities of muscular tone and movement the anatomical connections in the spinal cord between the afferent neurons of the peripheral nerves and the dendrites of cells, which make up the spino-cerebellar tracts, are not necessarily restricted to the proprioceptive system. While no disturbance of touch, pain, hot or cold sensation has been observed following cerebellar lesions neither is the conscious appreciation of proprioceptive sensation interfered with when lesions are restricted to the cerebellum. Nevertheless it was with some surprise that one of us (2) observed that electrical stimulation of the saphenous nerve in the cat gave rise to cerebellar action potentials. Snider and Stowell (7) briefly reported that in the cat tactile stimulation of the forepaw and other parts of the body produced action potentials in the cerebellum. Our observations in the rat were apparently made at the same time as those of Snider and Stowell for the two reports appeared simultaneously (5).

Our purpose has been to compare the cerebellar action potentials produced by "physiological" stimulation of exteroceptors with "physiological" stimulation of proprioceptors. At the same time we wished to observe possible differences in the responses when different parts of the body were stimulated. Admittedly the rat is not the most suitable animal for the latter part of the study. Previous trials in the cat had been discouraging and when these potentials were readily obtained in the rat studies in this species were continued. After reading Snider and Stowell's report we have confirmed the fact that light touch of the paw and the nose of the cat will also produce action potentials in its cerebellum.

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MATERIAL AND METHODS

The present study is based upon observations on 34 rats. They were lightly anesthetized with nembutal, 0.1 cc. of a 6 per cent solution per 250 g. Methods of stimulation were chosen which would give a fairly synchronous volley of impulses. For the proprioceptive stimulation the quadriceps femoris muscle in the hindlimb and the triceps brachii muscle in the forelimb were detached at their insertion and fastened to the animal board by a taut cord. The femur or humerus was fastened securely by a small specially made clamp and the remainder of the limb frequently denervated. Care was always taken that sufficient skin was removed from the extremity so that tapping the cord which suspended the muscle caused no movement of any part of the animal except the muscle itself. The cord was struck lightly, by a small metal rod attached to an electro-magnetic device which was activated at the proper sequence of each sweep of the beam of the cathode-ray oscillograph. This produced a synchronous excitation of stretch receptors. For the exteroceptive stimulation an ordinary wooden applicator was fastened to the above device so that it would sweep parallel to the surface of the animal's body in a cephalic direction but only close enough to move the hair of the back or proximal parts of the limbs. The skin itself was never touched nor was the slightest movement of the animal's body produced. In an active preparation only the tips of the longest hairs need be touched by the moving applicator to produce an action potential on the cerebellum. The amount of excursion of the device could be adjusted so that if desired a very localized patch of hair could be stimulated. In some experiments where the latency of the response was determined a "click" sound was produced by the electro-magnetic device. Although the presence or absence of this sound affected the action potentials led from the inferior colliculus no effect was ever seen on the cerebellar responses, nor was the "click" itself ever observed to produce any cerebellar response.

The lead electrode was usually a pointed cotton wick moistened with Ringer's solution and applied lightly to the exposed surface of the cerebellum. An indifferent electrode was applied to the skull. In a few experiments steel needles, insulated except at their tips, were used as lead electrodes. These were mounted in a holder 1 mm. apart. In no instance did the lobes giving responses with these "bipolar" electrodes differ from the lobes giving responses when explored by the single cotton wick electrode. Only the surface lobes which could be exposed readily were studied. These included the highest folium of the culmen, the lobulus simplex, the folium and tuber vermis, the pyramis, the uvula, Crus I and II of the lobulus ansiformis and the lobulus paramedianus. The basal parts of the anterior lobe, the paraflocculus and the flocculonodular lobe were not explored. In some experiments simultaneous records were taken from two points on the cerebellum and the responses from an identical stimulus compared. The action potentials were amplified by condensor coupled amplifiers and recorded by a cathode-ray oscillograph.

RESULTS

Stimulation of quadriceps femoris muscle. Cerebellar responses following stimulation of proprioceptive endings in the quadriceps muscle were studied in 18 animals. In seventeen of them potentials of significant amplitude were obtained when leading from the cerebellum. In twelve of these experiments the responses of greatest amplitude were recorded when the leads were on the pyramis, Fig. 1B. The potentials recorded from the pyramis were always surface positive while those from the other lobes explored were usually surface negative or diphasic. When surface positive potentials of large amplitude were lead from other lobes besides the pyramis they invariably appeared after a much longer latency than the pyramidal response. The most surprising finding was the almost complete absence of potential change when leading from the uppermost folium of the culmen. The deeper folia of the culmen were not explored. In certain experiments all the other lobules explored showed some activity. In some experiments responses were found only when

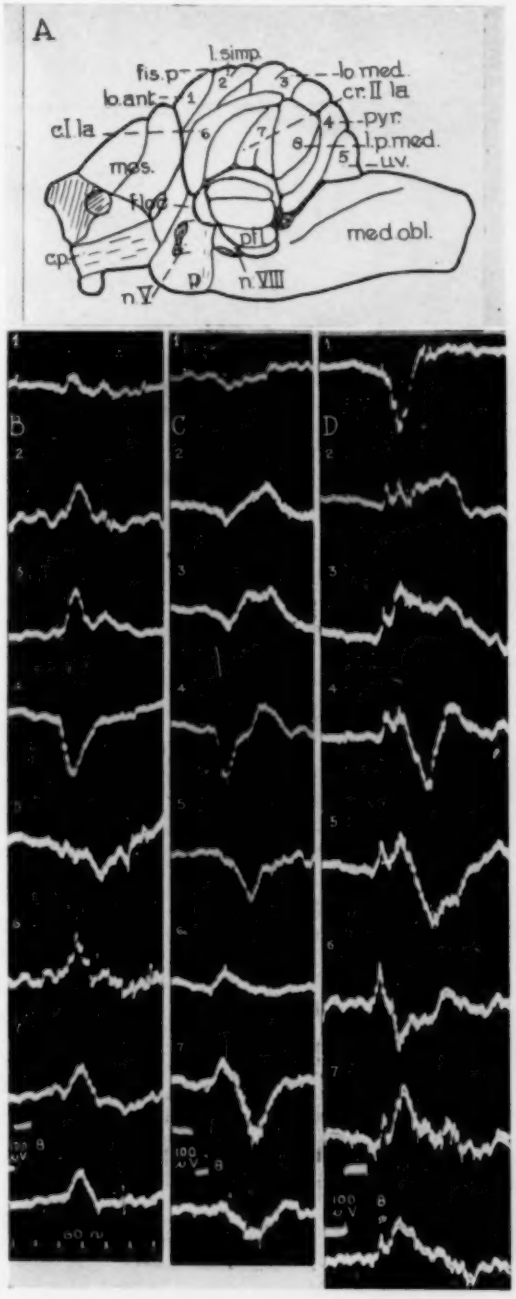

FIG. 1. A. Lateral view of the rat's cerebellum. Nos. 1 to 8 refer to the lead points for the corresponding records. c.p., cerebral peduncle; c.I. la., Crus I lobulus ansiformis; cr.II la., Crus II lobulus ansiformis; fis. p., fissura prima; floc., flocculus; lo. ant., lobus anterior (highest folium of culmen); lo. med., lobus medius (folium and tuber vermis); l. simp., lobulus simplex; l. p. med., lobulus paramedianus; med. obl., medulla oblongata; mes., mesencephalon (superior and inferior colliculi); n.V., trigeminal nerve; n.VIII, acoustic nerve; p. pons; pfl., paraflocculus; pyr., pyramis; uv., uvula. B. Potentials recorded following tapping the tendon of the homolateral quadriceps femoris muscle. A deflection downward in these and subsequent records indicated a positive potential. The instant of stimulation is not shown but all records in each column have been mounted so that the time sequence of one is comparable to another. Note an early positive potential only in the pyramis, B4. C. Potentials recorded following tapping the tendon of the homolateral triceps brachii muscle in the same animal. Note the largest early surface positive potential is led from the pyramis, C4. Other surface positive potentials of comparable amplitude are led from the uvula, C5, and Cruss II, C7. They occur respectively 17 and 21 msec. later than the one led from the pyramis. D. Potentials recorded following moving the hair on the back of the same animal. Note the early surface positive potentials are restricted to the culmen. Late activity is very widespread in this experiment which should be contrasted with the more usual distribution of activity as shown in Fig. 2 A.

leading from the pyramis, folium and tuber vermis and uvula. Potentials were never absent on the pyramis and present on any other lobule.

The latency of the response as measured from the time the tendon was struck to the beginning of the surface positive deflection on the pyramis was

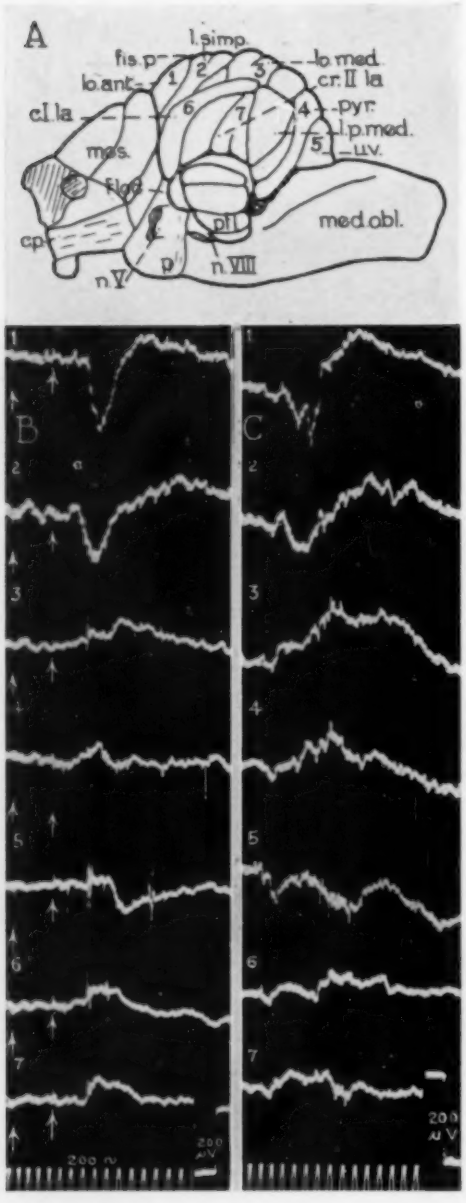

FIG. 2. A. Lateral view of the rat's cerebellum. Nos. 1 to 7 refer to the lead points for the corresponding records. B. Potentials recorded following stimulation by moving the hairs on the animal's back. The first arrow indicates the artifact caused by the electrical impulse which activates the electro-magnetic device. The beginning of actual stimulation is 5 msec. later. The second arrow indicates the end of stimulation. The deflections of short duration are artifacts caused by the electromagnetic device. Note the early surface positive potentials are found only on the upper folium of the culmen, B1, and the lobulus simplex, B2. C. Same lead points with single shock electrical stimulation of the homolateral sciatic nerve.

usually between 5 and 8 msec. This is significantly less than the figure given in the preliminary report of this work (5) but a more accurate method of determining the instant of stimulation eliminated a source of error which was responsible for the incorrect figure of 16 to 18 msec. previously given.

Stimulation of triceps brachii muscle. The triceps muscle was stimulated in 12 of the above 18 experiments and in two additional animals. In all but two experiments the responses of greatest amplitude were found when leading from the pyramis. These two exceptions were hyperactive preparations and in these animals potentials of greater amplitude were found on the lobulus ansiformis (Fig. 1, C7). The peaks of these potentials occurred 15 msec. after the peak of the potential recorded from the pyramis. In many of the experiments responses were confined to the pyramis, uvula and folium and tuber vermis. Indeed the only difference in the areas showing responses when the triceps was stimulated as compared to those when the quadriceps was stimulated was the occasional appearance of potentials on the culmen when the triceps was stimulated (Fig. 3, B2). Differences in latency were not always demonstrable between the responses from stimulation of the triceps and quadriceps muscles. In experiments in which the latencies from

the quadriceps were relatively long the triceps responses were detected about 3 msec. earlier than those resulting from the stimulation of the quadriceps.

Stimulation of hair. These experiments were done on 28 animals, 14 of which were also used for studying the effects of proprioceptive stimulation. The responses on the cerebellum resulting from this type of stimulation were more variable in their distribution, their sign and duration than was the case for either proprioceptive stimulation or electrical stimulation of peripheral nerves. In 8 experiments responses were detected on all the lobules of the rat's cerebellum, which we explored (Fig. 1, D). In 26 out of 28 animals the culmen showed the largest response and in many the only surface positive potential. In about half of the experiments a small surface positive potential was also seen on the lobulus simplex (Fig. 2, B). These deflections, whose duration was 15 to 20 msec., were usually followed by a long surface negative potential which lasted 50 to 75 msec. In some preparations (Fig. 2, B) this was practically the the only activity seen. Usually if any potential was seen coincident with the positive potential on the culmen it was a surface negative deflection (Fig. 1, D; Fig. 2, B). This m'ght in turn be followed, in active preparations, by a late surface positive or surface negative potential. Of particular interest was the contrast between the responses on pyramis and uppermost folium of the culmen when exteroceptive and proprioceptive stimulation was

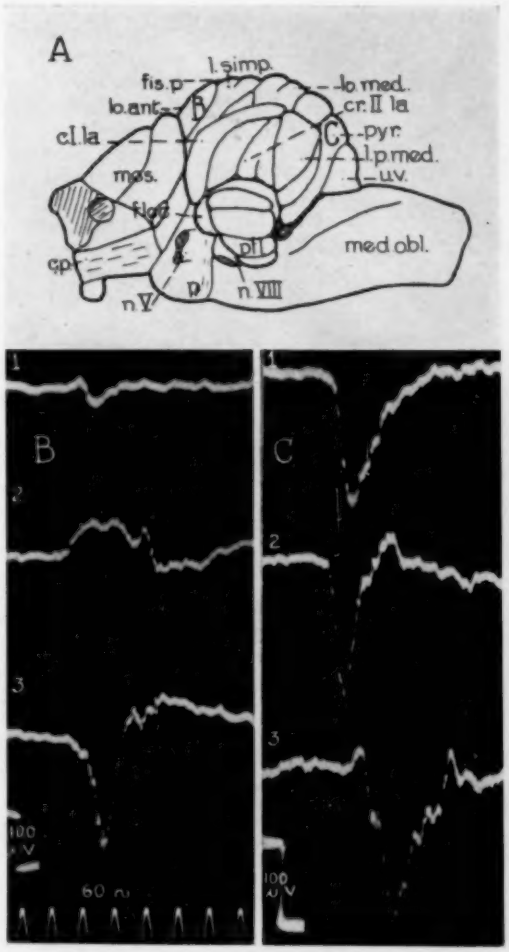

FIG. 3. A. Lateral view of the rat's cerebellum. Letters B and C indicate the lead points for their respective records. B. Records with lead on the highest folium of the culmen. Stimuli applied without changing lead electrodes. 1, tendon of the homolateral quadriceps femoris tapped; 2, tendon of the homolateral triceps brachii tapped; 3, hair on the animal's back moved. Stimulus artifacts are not shown but the records are mounted so that time sequence of each pair correspond. C. Simultaneous records led from the pyramis following the identical stimuli which produced those in B. Note that although a surface positive potential of considerable size is present in the pyramis record following stimulation of the hair, C3, it occurs 16 msec. after the culmen response.

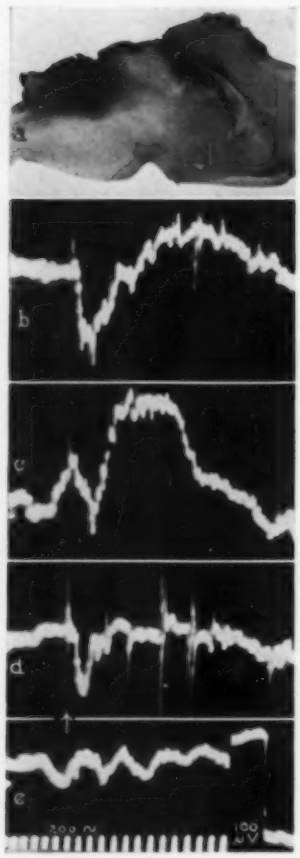

FIG. 4. a. Photograph of the mid-sagittal section of the rat's cerebellum following extirpation of all the cerebellum except the most caudal folium. The space ordinarily occupied by the central part of the cerebellum is filled by a blood clot. b. Response on the highest folium of the culmen following moving the animal's hair. c. Lead from the inferior colliculus before ablation of the cerebellum, with stimulation as in b. "Spontaneous" activity in the colliculus distorts the response. d. Lead from the inferior colliculus after ablation of the cerebellum; stimulation as in b. The movement of hair for the records b, c, and d was accompanied by a "click" sound which occurred at the arrow. Note the presence of a surface positive potential shortly after this sound stimulation. Same response was obtained with "click" alone without touching the hair. e. Same lead and same exteroceptive stimulation but without sound. Note the absence of a response from the inferior colliculus.

given. As may be seen in Fig. 3, proprioceptive stimulation of either the fore or hind limb causes more activity in the pyramis than in the culmen when a comparison was made with identical stimuli. With stimulation produced by moving the hair either the reverse occurred or as was the case in this experiment (Fig. 3) both pyramis and culmen are activated. It should be emphasized however that when the latter takes place the peak of the action potential in the pyramis appeared appreciably after that in the culmen; 15 msec. in the experiment shown in Fig. 3.

The responses to stimulation of the exteroceptors did not differ in any consistent way when the hair on different parts of the trunk or proximal parts of either homolateral extremity was moved. The latency of the responses following exteroceptive stimulation was difficult to determine because the stimulus itself was not instantaneous. It occupied 5 to 15 msec., depending on how large an area of hair was moved. The surface positive potential on the culmen appeared 20 to 30 msec. after the beginning of the stimulation and reached its peak 7 to 10 msec. later.

The peak of the late potentials occurred from 55 to 75 msec. after the beginning of the stimulation. These late responses were much more frequent following movement of the hair than in the stimulation of proprioceptive endings. Whether they represented the arrival of a long delayed afferent volley to the cerebellum or were the result of intracerebellar connections was not determined.

Because of the close proximity of the inferior colliculus to the anterior lobe of the cerebellum in the rat and the frequent bleeding at this site, following exteroceptive stimulation, an action potential could frequently be led from the inferior colliculus (Fig. 4, c). In order to be certain where the

potentials actually originated, decerebration and destruction of the inferior colliculus or the cerebellum was carried out at the end of many of the above experiments. Complete removal of the cerebellum always eliminated the responses on the inferior colliculus to movement of hair (Fig. 4, e). The removal of the cerebellum did not itself damage the colliculus. This could be shown by the fact that responses to "clicks" could still be detected (Fig. 4, d). Removal of the colliculus or decerebration at the level of the inferior colliculus did not interfere with the cerebellar potentials as long as the general condition of the animals did not deteriorate. Potentials identical in appearance to those found on the colliculus following exteroceptive stimulation could be detected if the lead electrode was applied to a pledget of cotton placed in the site of the extirpated inferior colliculus. It is our belief that all potentials recorded from the inferior colliculus following movement of the animal's hair originated in the anterior lobe of the cerebellum.

Electrical stimulation of sciatic nerve. Electrical stimulation of the sciatic nerve in the rat (Fig. 2, C), produced more widespread responses on the cerebellum than was the case in the cat (2), and much more so than in the monkey (3). Although it might be expected that electrical stimulation of a mixed nerve like the sciatic would give the sum of the responses produced by the proprioceptive and exteroceptive stimulation such was not exactly the case. In fact much to our surprise the distribution of the potentials resulting from this stimulation resembled closely that produced by moving the hair. Although in a given experiment responses to electrical stimulation might be of greater relative size on the pyramis than with exteroceptive stimulation (Fig. 2, B4 and C4), in none of the 5 animals in which the sciatic was stimulated electrically were action potentials found when leading from the pyramis which were as large as those produced by tapping the tendon of the triceps or quadriceps muscles. Whether or not this observation will be confirmed in a large series of animals remains to be seen. The responses in the vermian lobes began 5 msec. after the shock artifact. As was true of both exteroceptive and proprioceptive stimulation, late responses on the ansiformis were occasionally seen following electrical stimulation. When these were present they began 20 to 30 msec. after the shock artifact.

Discussion

A volley of impulses conducted toward the surface of the cerebral cortex is evidenced by an action potential of positive sign when a single lead is applied to the surface (1). If this is true for the potentials described above it would appear that of the cerebellar lobes explored in these experiments, the richest and most direct projection of fibers which may be activated by stimulation of proprioceptives is to the pyramis. Similarly, the culmen would represent the site of termination of most of the afferent fibers to the cerebellum which are activated by exteroceptive stimulation. These differences in the distribution of the early surface positive responses raises the possibility that the spinocerebellar tracts may differ in the modalities of sensation which each carries to the cerebellum.

Grundfest and Campbell (6) reported that electrical activity is initiated in the dorsal spinocerebellar tract by stimulation of nerves supplying muscles of the hindlimb but not by stimulation of the saphenous nerve. They found the cerebellar lobules activated by the dorsal spinocerebel ar tract to be in the basal folia of the anterior lobe. These folia are difficult to expose in the rat and it is not known whether they are activated by exteroceptive or proprioceptive stimulation in this species. A study by Marchi technique of possible differences in the termination of the dorsal and ventral spinocerebellar tracts in the rat is now being made. Snider and Stowell's (7) success in detecting action potentials in the cat's cerebellum in response to tactile stimulation opens the possibilities for further study of these differences in a species with a larger cerebellum and one more suitable for operative procedures on the cerebellar peduncles.

Our failure to find differences in the cerebellar action potentials depending on the topographical regions stimulated is not surprising in view of the fact that electrical stimulation (2) also failed to show any such differences. This is in accord with the lack of segmental differences in the termination of the spinocerebellar tracts as determined by experimental anatomical methods. (For a review of anatomical data bearing on this see 4.) However, the rat because of the small size of its cerebellum is probably not as suitable as some larger animal would be for the study of possible differences in the connections from topographically different parts of the body. Snider and Stowell (7) state that in the cat such differences exist and that it is possible to construct a map of the cerebellum showing the arrangement of the representation of different parts of the body. This is the first evidence of clear cut topographical localization in the cerebellum in respect to its afferent connections. If consistently confirmed it is an observation of first magnitude.

Summary and Conclusions

1. Cerebellar action potentials in the rat were recorded from surface folia following stimulation of exteroceptive receptors by moving the hair on different regions of the animal's body and of proprioceptive receptors by tapping the tendons of isolated muscles of the fore and hind limb. The lobules explored were the highest part of the culmen, the lobulus simplex, the folium and tuber vermis, the pyramis, the uvula, Crus I and Crus II of the lobulus ansiformis and the lobulus paramedianus.

2. Of the lobules explored the pyramis showed the most consistent activity following proprioceptive stimulation and the culmen following exteroceptive stimulation. There were marked differences in distribution of the responses depending on which type of stimulation was used. Some of the potentials may have resulted from activity caused by intracerebellar connections.

3. No consistent differences in the distribution of the responses was detected when different parts of the animal's body were stimulated. Because

of the small size of its cerebellum, the rat is not the most suitable animal for this study.

4. In respect to the cerebellar lobules activated, electrical stimulation of the sciatic nerve resembled exteroceptive stimulation more closely than proprioceptive stimulation. The distribution of activity throughout the cerebellum following electrical stimulation of the sciatic nerve in the rat was more widespread than in the cat, and much more so than in the monkey.

The authors wish to express their thanks to Mr. Fred Claussen for technical assistance.

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A COMPARISON OF EFFECTS OF UPPER AND LOWER MOTOR NEURONE LESIONS ON SKELETAL MUSCLE

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THE "WASTING" of paralyzed muscles has been recognized since antiquity. It received mention in the Bible. John Hunter, in his Croonian Lectures (6), stated the fact as one generally familiar. Differentiation between paralysis due to spinal trauma and that caused by peripheral nerve injury was implied by Pott (7) in the description of the disease now bearing his name. Barlow (1) recognized the main characteristics of the two types of paralysis. Essentially modern views on the subject were current by 1884 when Bramwell (2) wrote, "Where the lesion is situated above—the multipolar nerve cells of the anterior cornu the paralyzed muscles may be—moderately wasted (atrophy of disuse). Where the multipolar nerve cells are acutely destroyed—rapid atrophy results."

In recent times there has been a considerable body of clinical observation covering this field. The experimental approach was relatively neglected until Tower (10), using dogs, demonstrated atrophy in muscles connected to an isolated section of the spinal cord. This part of the cord was severed from the rest of the nervous system by cutting the dorsal roots and performing a complete transection of the cord itself above and below the region involved. Later, Tower, Howe and Bodian (11) found that a similarly produced atrophy in monkeys was not accompanied by fibrillation. Eccles (4), using cats subjected to the same experimental procedure, investigated the contractile properties of such neurologically isolated muscle and confirmed Tower's findings with relation to the atrophy.

Using albino rats we (9) have demonstrated marked atrophy in hind limb muscles due to high-section of the spinal cord. Such section does not "isolate" the involved segment of the cord, as with Tower's technique, but does abolish the upper motor neurone control of the muscles of the lower limb. Tower's method produces neurological isolation of the muscles being studied while the simple cord section removes higher control but leaves the spinal reflex arcs intact. The present experiments were undertaken to compare atrophy, fibrillary activity and acetylcholine sensitivity of muscles deprived of their upper motor neurone control with these properties in muscles deprived of lower motor neurone control, the so-called denervated muscles.

METHODS

Albino rats weighing between 200 and 350 gm. were used throughout. They were

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maintained in separate cages in a well-heated room at a fairly constant temperature. An adequate diet was available to all animals. Under ether anaesthesia the vertebral column was exposed and complete section of the spinal cord performed at about the level of the 6th thoracic vertebra. At the same time, denervation of the right gastrocnemius-soleus muscle group was effected by removal of a length of the sciatic nerve on that side. In animals which were to be kept for long periods of time, the completeness of this denervation was checked during the 3rd or 4th week.

In the initial experiments the immediate mortality, and that during the first few post-operative days, was high. This was lowered appreciably, however, by maintaining the animals in a room kept at a fairly constant temperature (about 80°F.). The animals appeared normal anterior to the mid-thoracic region but, of course, were incontinent and dragged their lower extremities. When spinal shock had passed off, *i.e.*, within a day or two, the lower limbs on the two sides revealed gross functional differences. The right was limp, whereas the left showed an immediate mass flexor reflex response when it was pinched but appeared relaxed except when thus stimulated. At various intervals after operation the animals were sacrificed, the fresh weights of both gastrocnemius-soleus groups determined, and each animal weighed.

In most cases, just prior to autopsy, the animals, under light ether anaesthesia, were subjected to the following procedure. With the limb immobilized, the tendon of the muscle group of one or other side was dissected out, detached from its insertion, and attached to an isotonic lever for recording on a smoked drum. In many but not in all cases the intact sciatic nerve was severed at this time to abolish reflex movements and thus simplify the experimental procedure. The action potentials were led from the muscle belly through two fine needles and amplified. They were recorded photographically, using a Matthews oscillograph, and rendered audible through a loudspeaker. Injections of acetylcholine in various dilutions but constant volumes (0.25 cc.) were made into the iliac artery, and the minimal effective dosage determined. Normal control animals of comparable weights were subjected to the same procedure.

Results

In order to simplify description, the muscle or limb on the right side will be referred to as "denervated," that on the left side as "plegic," and those in a normal control animal as "normal." The word "plegic" is derived by deleting the prefix "para," inapplicable in this case, from the familiar term "paraplegic." It should be noted that the so-called "plegic" muscles showed no evidence of activity except as a reflex response to noxious mechanical stimulation or as a direct response to other forms of stimuli.

1. *Atrophy*. In all, 83 animals were used and killed at various intervals after operation. Those sacrificed in the first two or three weeks had lost considerable weight, but the later animals showed a subsequent gain.

During the first two weeks, the gastrocnemius-soleus muscle groups in any one animal showed practically identical weight loss on the denervated and plegic sides. Differences up to 10 per cent were encountered between the weights of the muscles on the two sides in occasional animals, but such deviations showed no consistency in either direction.

Figure 1 shows graphically the course of muscle weight loss in these animals. As in our previous paper (8), atrophy is represented by the decimal fraction of the ratio D/I. D is the fresh wet weight of the atrophic muscle, and I the weight of the corresponding normal muscle. In the present series, an estimation of a value for I was made on the basis of the animal's body weight at autopsy. This seems justified as, in a large group of normal animals, the gastrocnemius-soleus muscles bore a fairly constant ratio (1:65) to body

weight. The estimated atrophy was probably less than actually took place, as there was a general loss of body weight. However, the progressive and parallel courses of the atrophic processes are apparent from this graph. For comparison, the course of atrophy following denervation in otherwise normal animals is included in the figure. Here, of course, D and I are actual determinations of the weights of the denervated and normal muscles.

Between the second and third weeks after operation a change became apparent. The animals continued to drag their hind limbs, remained incontinent, and appeared generally comparable to those sacrificed earlier, which led us to believe that no effective repair of the cord lesion had taken place.

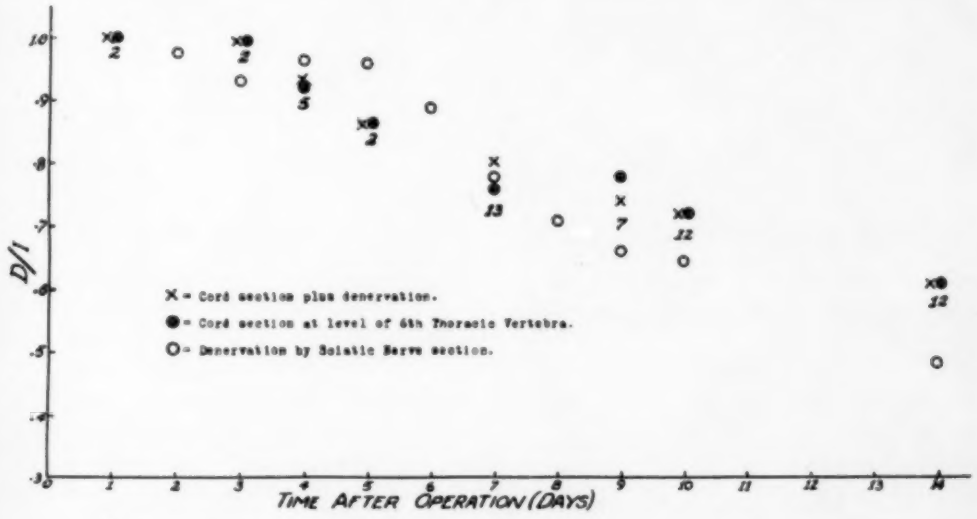

FIG. 1. Muscle atrophy following the operation indicated in the legend. The figures beside the points indicate the number of animals in the "Cord section plus denervation" and the "Cord section at level of 6th thoracic vertebra" series.

However, as time went on, a difference in the size of the muscle-groups on the two sides became increasingly evident. The weight loss progressed in the denervated muscles, whereas recovery took place in the plegic side. In Fig. 2, the time scale of Fig. 1 has been reduced and the duration increased to show graphically the course of this divergence. The plegic muscles, deprived of upper motor neurone control, recovered rapidly from the initial marked atrophy and, in the longer experiments, almost regained their normal size and appearance. In the denervated muscles, however, atrophy advanced steadily until, at the end of 8 weeks, little more than thin bands of contractile tissue remained.

The muscle groups of several animals were examined histologically in paraffin sections. In the first 2 weeks, both showed a shrinking of muscle fibres and proliferation of sarcolemmal nuclei but the fibres retained their striations. Later, however, the fibres of the plegic muscles recovered a nor-

mal histological appearance, whereas the denervated muscles progressed to typical atrophy. Good nerve endings were readily demonstrable, with gold technique, in the plegic groups, whereas repeated attempts failed to demonstrate any such endings in the denervated preparations.*

2. *Action potentials.* When these animals were tested at any time after the 3d postoperative day, the typical action potentials of fibrillation were readily detected on the denervated side. These were at a maximum between

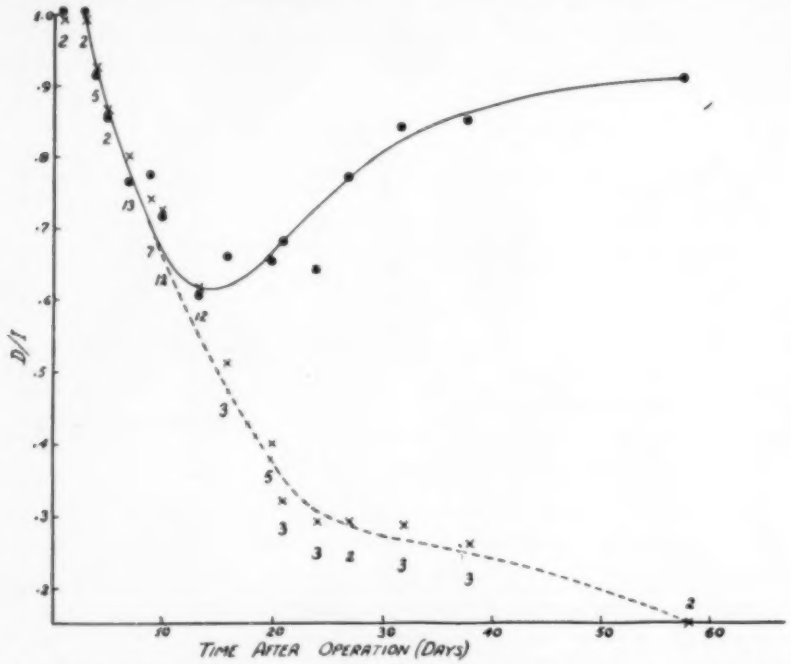

FIG. 2. An extension of Fig. 1 showing muscle atrophy following "Cord section at level of 6th thoracic vertebra" (•) and "Cord section plus denervation" (×). The figures besides the points indicate the number of animals contributing to the averages represented by the points.

the 6th and 14th days, but subsequently decreased in amplitude. After 8 weeks, though much diminished, they were still detectable. These action potentials of the denervated muscle were not modified by even vigorous pinching of the limb, but were readily abolished by quinidine (8).

On the plegic side, under the light ether anaesthesia, no activity was observed at rest. In response to mechanical stimulation, an immediate outburst of action potentials resulted. This outburst, the electrical concomitant of the mass reflex response described above, could be elicited 2 days after operation and was detectable even 8 weeks later. It could be abolished by section of the sciatic nerve but was unaffected by doses of quinidine which would abolish the fibrillation of denervated muscle.

* We are indebted to Professor E. A. Linell and Dr. M. I. Tom of the Department of Neuropathology for this examination.

3. *Acetylcholine sensitivity.* Brown (3) stressed the marked sensitivity of denervated skeletal muscle to the intravenous or intra-arterial injection of acetylcholine. In the rat, it has been shown that the denervated gastrocnemius-soleus group shows a minimal response to the intra-arterial administration of doses of 0.002–0.02γ of this material. On the other hand, only dosages of 200γ–2.0 mg. were effective in stimulating comparable normal muscles. In the present experiments, a similar comparison was made between the denervated and plegic muscles at various stages in the course of atrophy depicted in Fig. 2. For the first 2 or 3 days, the muscle groups on both sides behaved as normal muscles in response to acetylcholine injections. The lower record of Fig. 3, which shows the response of such a normal muscle, is the only one of 30 experiments where 200γ of acetylcholine produced any mechanical movement. In the remainder, 2 mg. doses were necessary to be effective.

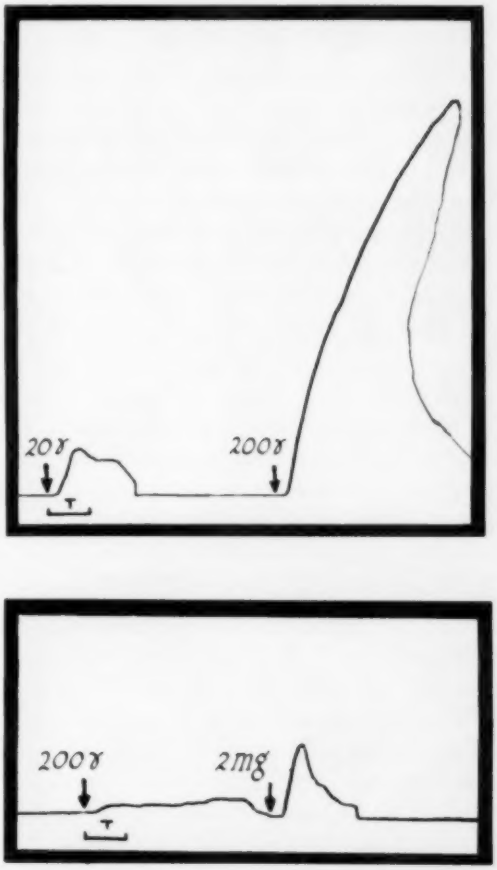

Fig. 3. Mechanical responses of gastrocnemius-soleus muscles of rat to intra-arterial injections of acetylcholine at points indicated by arrows.

Upper record shows the reaction of muscle with upper motor neurone lesion to injections of 20γ and 200γ of acetylcholine.

Lower record shows the reaction of normal muscle to 200γ and 2 mg.

T = 10 sec.

Commencing at the third day after operation, however, the denervated muscles showed an increasing sensitivity to acetylcholine which was at its maximum by the 6th or 7th day. These muscles were stimulated by doses of 0.002–0.02γ administered intra-arterially. It was difficult, in the animals tested several weeks after operation, to be certain of minimal mechanical movements in the presence of such marked atrophy. However, it appeared that even after 8 weeks this increase in sensitivity to acetylcholine was still present.

Again commencing at the 3d day, a much smaller, but nevertheless definite, change in sensitivity was exhibited by the plegic muscles. Approximately 20γ of acetylcholine produced a response in this group of muscles. Not only were the muscles stimulated by doses which were thus 10–100

times lower than those necessary for normal muscles, but, as seen in Fig. 3, larger doses produced responses of greater magnitude than were ever seen with normal muscles. It was suspected at first that these results might be explained by destruction of some anterior horn cells in the initial operative procedure. In this event a partial denervation of the muscle might have been produced without being extensive enough to produce fibrillation detectable by our technique. However, cordotomy at levels well above and below the site usually chosen produced no fibrillation, and the increased sensitivity of the plegic muscles was unchanged. These findings indicated that it was most improbable that the lowered threshold to excitation by acetylcholine was caused by undetected lower motor neurone damage. Furthermore, this altered threshold only persisted during the first 2–3 weeks postoperatively. In animals tested after the 20th day, it was found, with 2 exceptions, that the spastic muscles responded only to the larger doses which were effective with normal muscles (2.0 mg.). The exceptions were found on the 24th day (Fig. 2) when, in two of three animals tested, the plegic muscles showed the lower threshold and increased response to acetylcholine described above. It is to be noted that the atrophy of these particular muscles was a good deal more marked than one would have expected.

Discussion and Conclusions

In the experiments reported in this paper, the muscles of the lower limbs were deprived of normal upper motor neurone control by the transection of the spinal cord in the mid-thoracic region. This was modified by cutting the right sciatic nerve, in this way producing lower motor denervation on that side. Such animals showed an intact reflex arc and normal motor nerve endings on the left side. On the right side the denervated muscles demonstrated the typical changes of nerve degeneration with muscle fibrillation. In the first 2 weeks postoperatively, there was an equal and parallel atrophy in the gastrocnemius-soleus muscle groups of the two sides. Between the second and third weeks, a divergence appeared. Whereas the denervated muscles continued to lose weight steadily, those with intact motor nerves began to show a gradual recovery of muscle bulk which progressed until, in some cases, an approximately normal muscle weight was attained. Following the preliminary report (9) outlining this development and regression of atrophy Fischer (5) described experiments supporting these findings.

Throughout our experiments the animals presented the same general appearance, dragging the lower half of the body and remaining incontinent. Furthermore, the readily-elicited mass reflex response on the left side remained unchanged and there was no apparent increase in muscular activity to account for the regression of atrophy. Were the atrophy the result of a temporary damage to some or all of the anterior horn cells at operation, it would seem unlikely that the mass reflex would remain unchanged throughout the whole course of the experiments. Operative damage to such cells, if of any extent, would produce a permanent, rather than transient, muscle

weight loss and one would expect to detect fibrillation. The plegic muscles always showed regression of atrophy and never exhibited fibrillation. Furthermore, if anterior horn cell damage were the cause of the atrophy a cordotomy performed several segments lower should prolong the atrophy or render it permanent. The onset and regression of atrophy were unchanged when this was done.

The plegic muscles were apparently, apart from occasional reflex spasms, at rest. They showed no prolonged spastic state detectable by our methods and were certainly not subjected to as regular a bombardment by motor nerve impulses as are normal muscles. Disuse, relative or absolute, appeared to be a constant factor throughout the whole course of the experiments as judged by the behaviour of the animals. It is possible, however, that there was a low level of reflex activity in these muscles producing a slight steady contraction not noted on manipulation. Such an assumption in no way helps to explain the bizarre course of the atrophy.

Perhaps the onset and subsequent regression of atrophy in the case of an upper motor neurone lesion and onset and subsequent accentuation of atrophy in the case of a lower motor neurone lesion indicates the activity of two etiological processes. It is possible that the first short-lived process is seen in action in both types of damage but the second is active or is unopposed only in the case of denervated muscle. Knowledge of the cause of the regression of atrophy in the case of an upper motor neurone lesion may yield the key to effective treatment of denervated muscle.

The experiments of Tower (10, 11) and Eccles (4) in which muscles are left with an intact lower motor neurone but are protected from motor nerve impulses by isolation of the involved section of the spinal cord probably differ fundamentally from our experiments. In the one case the muscle is neurologically isolated and in the other the reflex arc is intact and the muscle is subjected to occasional large bursts of impulses producing massive contractions, possibly also, as previously suggested, to a steady flow of impulses producing a level of constant activity too small to be detected by our methods. Neither Tower nor Eccles attempted to follow the course of atrophy in their experiments and it is impossible to tell whether their animals showed the onset and regression of atrophy seen after simple cord section. In both types of experiments pyramidal (upper motor neurone) and extrapyramidal motor tracts are cut. While the former are predominant in their effect on muscular activity it is possible that simultaneous section of the latter modified the atrophic process. However, we know of no clear-cut evidence, either experimental or clinical, indicating that spinal cord tracts other than the pyramidal have a trophic influence on skeletal muscle.

In the present experiments a response in the muscles with intact motor nerves was elicited by smaller doses of acetylcholine than in normal muscles. However, this increased acetylcholine sensitivity was only seen during the period of atrophy. As recovery commenced, the muscles showed return to a normal degree of excitability. In the case of the denervated muscle greatly

increased sensitivity to acetylcholine appeared early and remained throughout the course of the experiment. These findings indicate that atrophy accompanied by increased sensitivity to acetylcholine may be produced by upper as well as lower motor neurone lesions and suggest that this picture is not exclusively a result of motor nerve ending or lower motor neurone destruction.

Summary

Using albino rats the atrophy of the gastrocnemius-soleus group of muscles following an upper motor neurone lesion (produced by spinal cord section at the 6th thoracic segment) was compared with the atrophy following a lower motor neurone lesion (section of the sciatic nerve). The two types of lesions produced atrophic processes which were quantitatively similar for the first 14 days. Thereafter the muscle groups with severed upper motor neurones started to recover lost weight while those with lower motor neurones cut showed an uninterrupted development of atrophy. Both types of injury produced hypersensitivity to acetylcholine in the muscles involved. This lasted only as long as the atrophic process was active and was most marked in the cases of section of the lower motor neurone. Fibrillation was seen only when the lower motor neurone was cut.

Possible causes of the onset and regression of atrophy in skeletal muscle deprived of upper motor neurone control are discussed.

It gives us pleasure to acknowledge the interest taken in this work by Professor C. H. Best.

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STIMULUS FREQUENCY AS A MEANS OF ANALYZING SYNAPTIC ACTIVITY

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CONSIDERING that many sense organs activate the centres by long trains of impulses it is surprising how little is known about the central effect of this mode of stimulation. In this laboratory Bernhard and Skoglund (2) have shown that a transient reflex pattern can be evoked in cats by restricting stimulation of certain nerves (*e.g.*, the popliteal) to certain frequencies. It consists of late widespread extensor contractions in all limbs, rise in blood pressure, pupil dilatation, etc., is absent below frequencies of 50 per sec., maximal around 100 per sec., lasts some 20–30 sec. and then disappears despite continued stimulation, even at optimal frequency. Their result shows that stimulus frequency may be a means of selecting certain reflex patterns and is of interest in view of the fact that this frequency range is well within the limits of normal sense organ activity.

Gasser's laboratory (9, 13, 10) has supplied us with important information about the effect of stimulus frequency upon peripheral nerve, and Lorente de Nó and Graham (20) have shown that the summation of subnormality known from peripheral nerve and augmented by repeated stimuli, is of significance in the recovery cycle of motoneurons. These studies have drawn attention to the depressing effects of frequency, analogous in peripheral nerve and centres, but there must also be slow facilitating effects to account, for instance, for slow "recruitment" (16).

Depression of synaptic activity, "recruitment" and selective effects of frequency are phenomena which also have turned up in this work, which is devoted to preliminary observations on the effect of stimulus frequency on direct and relayed waves caused by stimulation with electrodes buried in the spinal cord when the response is recorded from the sciatic nerve (22, 17). Prior to analyzing the effect of long trains of impulses under separately evoked facilitating and depressing influences it was deemed necessary to possess some information about the effects of frequency as such on direct and relayed waves.

METHOD

Stimulating electrodes have been a pair of needles, at a distance of 2 mm., insulated except at the tip, thrust into the spinal cord of decerebrate cats through the *unopened* dura in the lumbal region, generally in a ventro-lateral position, sometimes in the dorsal root region. The sweep circuit operating through an amplifier has been connected through a compensator bridge to these electrodes. A maximal rate of 850 per sec. could be obtained by these means. The strength of the stimuli was independent of rate.

In most experiments the leads were on the sciatic nerve, one of them on the crushed end, and connected to a balanced condenser-coupled amplifier.

The sweep circuit drove the beam of the cathode ray horizontally at each stimulus and the film was passed slowly at right angles to the sweep. Now and then the film was

stopped and several records superimposed, so as to give an average picture of the effect. Rather slow speeds had to be used to save film. As the sweep was linear no time marking was necessary and the total width of each picture, being inversely proportional to stimulus frequency, measured time directly. As a consequence the events recorded are expanded horizontally by an increase in stimulus frequency.

The cat was surrounded with vapour from a layer of water covering the bottom of the screened animal-box heated from below. The rectal temperature was around 37.5–38.5°. The animal was undenervated with the exception of the nerve to be recorded from, as our intention was to allow a background of free play of impulses from the natural sources.

Results

1. *General description of response.* Confirming Renshaw (22) and Lloyd (17) we find the direct spike wave caused by stimulation of the ventral horn cells or their axons, succeeded by one or several relayed waves. The direct wave is easily recognized by measuring its latent period which is practically equal to the conduction time at alpha velocity (around 80–100 per sec.). Simpler still is to diagnose it by stimulating with a high frequency. As is to be expected from the work of Gasser (9) direct waves generally follow the whole range available in the stimulator (350 per sec.) whereas relayed waves disappear far below this value. By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained. These, as we shall see, vary from case to case. The necessity of having a direct wave has restricted us to stimulation of places giving such waves (17) or to crossed stimuli symmetrically placed on the opposite side of a place for which the direct waves have been measured previously.

By means of Fig. 1, a typical experiment will be surveyed. The recording electrodes are on the undivided sciatic nerve. Stimulation begins in A with frequency 70 per sec. After 20 impluses the film is stopped for a moment. Two large waves are then clearly seen; the first, the direct wave with an average latent period of 0.9 msec., the second a relayed wave with an average latency of 3.0 msec. The central reflex time of the relayed wave is thus 2.1 msec., assuming that it is conducted in the same fibres as the direct wave. But going back in the same record A upwards there can be seen a second relayed wave, best marked in the beginning and fading out as stimulation proceeds. Its latent period is 5.7 msec. Subtracting the direct wave makes the central reflex time of the second relayed wave 4.8 msec., still assuming it to be conducted by the fibres conducting the direct wave. This assumption is a necessary approximation.

Record B of the same figure corresponds to stimulation frequency 100 per sec. Both relayed waves are seen in the beginning, the first alone when somewhat later (bottom of record) the film is stopped for a moment in order to make a good print. This frequency is the limiting frequency for the second relayed wave. It is, of course, possible to push the limen somewhat higher. For practical reasons it is, however, easier to take as the limiting frequency a value for which in the beginning of each record there are still traces left of the wave to be analyzed, say, for the first three to

five stimuli. Using this criterion the relayed wave with the central reflex time 4.8 msec. possesses the limiting frequency 100.

In the next record C, at stimulus frequency 140 per sec., only the direct and the first relayed wave are seen, further apart now, while this frequency means that the sweep traverses its path in 7.15 msec. as against 14.3 msec. in record A. The width of the sweep path in msec. is obtained from the inverse value of the frequency. In record D the frequency is 198 per sec. The first relayed wave still persists though now pushed near the end of the

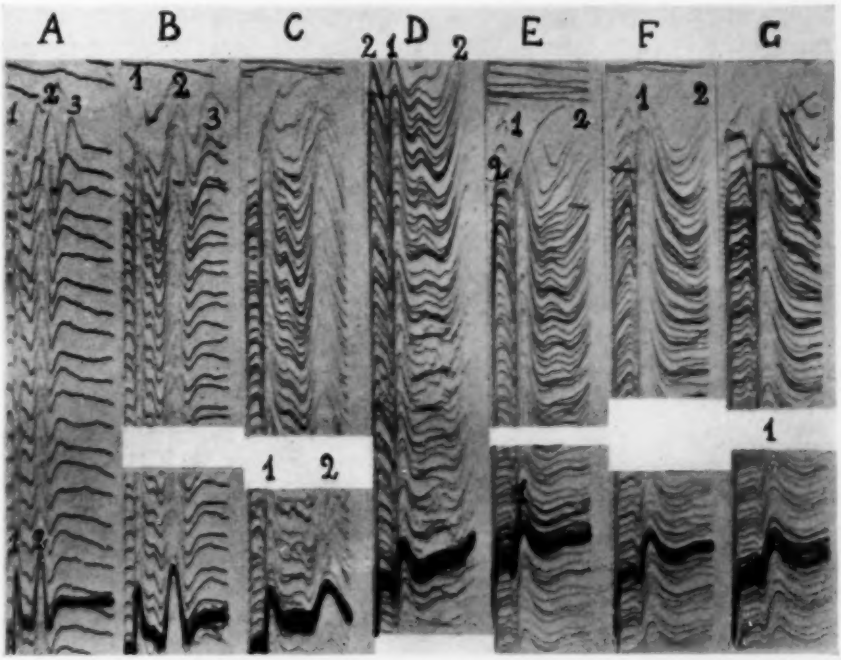

FIG. 1. Leads on the sciatic nerve. Bipolar stimulating needle electrodes in ventrolateral position 3 cm. above the cams of the cristae. Beginning of each record shown for different stimulation frequencies. Later in each record the film has been stopped to show some waves superimposed to give a good print. Full description of experiment in text. To be read downwards. In this and all records, 1, direct wave, 2 first, 3 second relayed wave.

picture. Its latent period is shorter in the beginning of the record and lengthens downwards. In record E, at frequency 230 per sec., the first relayed wave is present only in the beginning. In record F the frequency is 268 per sec. and this is the limiting frequency for the first relayed wave with central reflex time 2.1 msec. which is still seen in a few initial beats. In the last record G the frequency is 310 per sec. Only the direct wave is seen. At this frequency the greater part of the relayed wave would have passed over the shock artifact to reappear in the beginning of the sweep, if present. In records D, E, and F it is seen to have done so.

The experiment shows that the limiting frequency is reduced by a

lengthening of the central reflex time and that, as stimulus frequency increases, the relayed waves disappear, so to speak, in good order and not by getting lost in general desynchronization. An essentially constant response pattern (but see below) is kept up until the limiting frequency is reached. During continued stimulation at higher frequencies the waves rapidly flatten out and disappear.

Figure 2 is introduced to show another type of pattern in which the direct wave is large and the first and second relayed waves are small because the stimulus is placed low in the cord. At slow speeds, A and B, the fast direct wave merely appears as a gap in the path of the sweep, but its size is shown late in record B when the film has been stopped for a while.

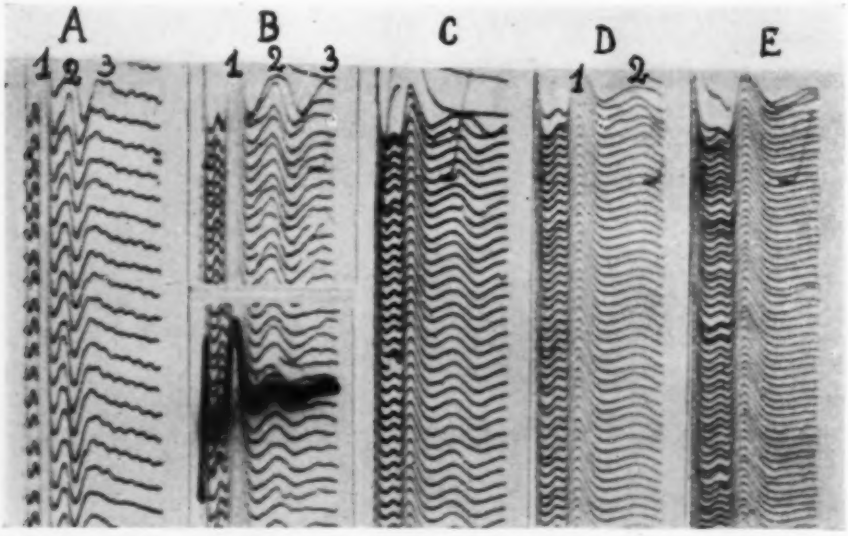

FIG. 2. Leads on the popliteal nerve. Bipolar stimulating needle electrodes just above the cams of the cristae. A, at 100 per sec.; B, 170 per sec.; C, 230 per sec.; D, 268 per sec.; E, 310 per sec. Limiting frequency between D and E, nearer to the latter. To be read downwards.

Figure 3A shows some portions of the course of the typical diminution of the two relayed waves of a response caused by repetition of the stimulus. The frequency is 140 per sec. This diminution which need not be noticeable for several seconds at lower frequency is also determined by the strength of the stimulus. Thus in Fig. 3, at 100 per sec., the stimulus is strong in record B and weaker in record C. The relayed wave decreases rapidly in C and not at all in B. The film is stopped after practically the same number of stimuli in both B and C, taken in succession with stimulating and recording electrodes in the same position. Record D of Fig. 3 illustrates the often relatively complex response of crossed stimuli to the spinal cord. It may, however, be much simpler. Records E and F in another experiment, taken at different rates of stimulation show the first wave to possess two peaks.

The first peak is the direct wave, as measurements show, but how about the second peak in this case? The frequency test showed it to be present at rates (600 per sec.) as high as to be above any values seen with other relayed waves. It is, at least at this stage of our work, safer to regard secondary waves of this character as direct waves delayed by slower conduction rates in the motoneurones concerned.

Evidence for dispersion of the relayed waves is found in most records (22, 17). They are nearly always wider than the direct waves meaning that the synapses themselves differ in kind or number, that the diameter and length of the pathways differ or that facilitation in the short proprio-

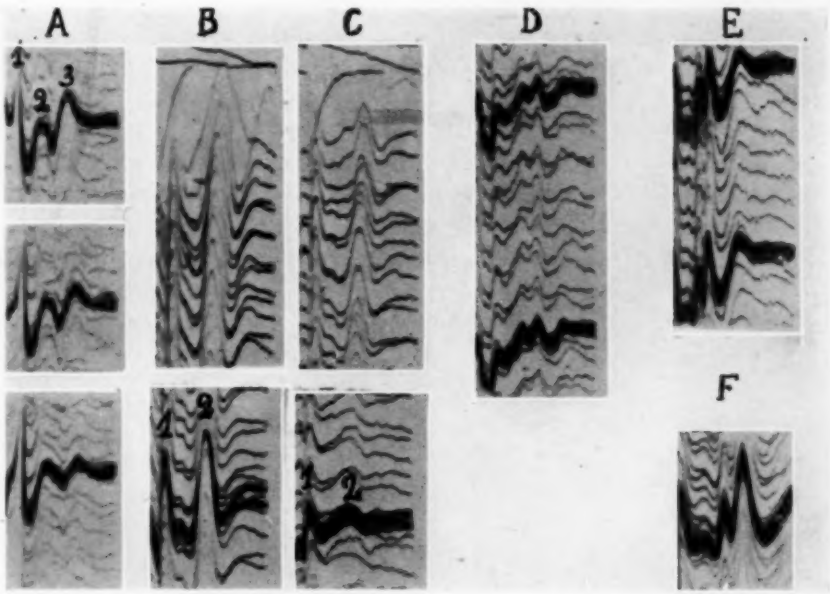

FIG. 3. A, leads on the peroneal nerve. Continued stimulation of spinal cord at 140 per sec. to illustrate typical decrease of relayed waves. B and C, leads on the sciatic in another experiment. Stimulation at 100 per sec. Stimulus strong in B, weak in C. Record D from another similar experiment shows the complex response often obtained when the stimulating electrodes have been placed on the contralateral side of the spinal cord. E and F from another experiment with the usual homolateral stimulation of ventrolateral side of spinal cord at frequencies 100 (E) and 198 (F) per sec. See text. To be read downwards.

spinal neurones (17) comes on slowly. The potentials set up by the simultaneously active and by the anatomical situation best synchronized synapses probably also activate adjacent elements electrotonically thus contributing to the dispersion. "Grouped action" of this type is very characteristic for motor neurones (23) and probably means a great deal for the characteristic grouping seen here. Nevertheless it is clear that the direct wave does not leave room for the same amount of temporal spacing around the top and thus represents stimulation of a more uniform system.

2. *Recruitment.* A relayed wave which is fully developed from the beginning generally decreases in size during continued stimulation (cf. Fig. 3), provided that the frequency be sufficiently high. But quite often relayed waves are encountered which actually *in*crease in size as a consequence of repetitive stimulation so that the total wave pattern is better developed after some time than in the beginning of a record. This result signifies that new neurones have been recruited into activity, partly perhaps also gradually improving synchronization. Lloyd (17) has studied the effects of facilitation to two or three shocks and also points out that the effect refers to grouped discharges with characteristic central reflex times.

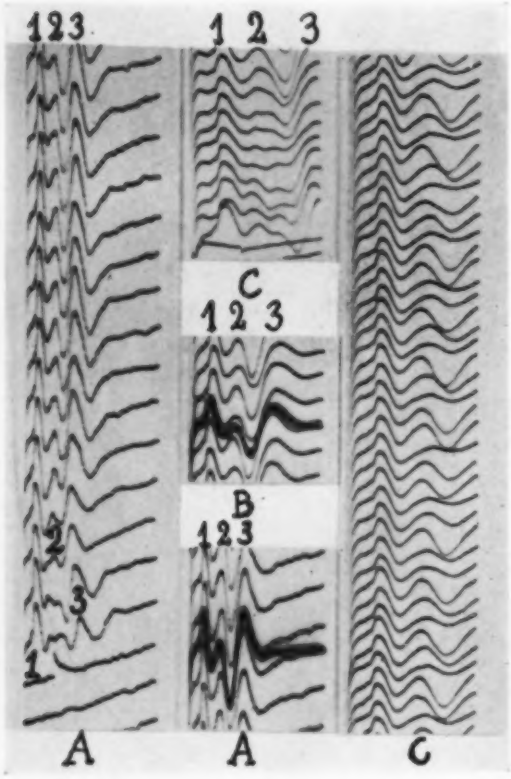

FIG. 4. All records to be read *upwards*. Record A, at 70 per sec. fully described in the text, continued into second row to show the fully developed effect with first and second relayed waves maximal and standing film. B, at 100 per sec. C, at 140 per sec. directly continued into third row. Full description in text.

Figure 4 illustrates a typical case. It begins with record A (to be read upwards) at frequency 70 per sec. In the beginning only the initial direct wave with latent period 1.0 msec., followed by the second relayed wave with latent period 3.8 msec., are clearly defined. Gradually a small wave becomes better visible between them. This is the first relayed wave with latent period 2.4 msec. The direct wave is of practically constant size throughout the record, but both the first and the second relayed waves increase in size up to about the 9th stimulus. At this size they were seen to remain during stimulation for a couple of seconds. A late portion of the film is found in the second row (A). Record B is around the 60th stimulus at rate 100 per sec. All three waves are clearly visible. Record C, continued in the third row, shows the beginning of the film taken at stimulation rate 140 per sec. The second relayed wave is now quite well developed from the beginning, but the first relayed wave is broad and not definitely set off from the rest until the 9th stimulus has been reached. The same record, continued in the next row, illustrates almost regular variations in the size of the relayed waves of the kind quite often seen at high frequencies. The

interesting point here is that the first and second relayed waves always vary in parallel, indicating a common influence on both of them, reminiscent of the intermittent conduction described by Barron and Matthews (1), rather than of the alteration seen at high frequencies in peripheral nerve. The latent periods of the three waves are here: 1.4, 2.9, and 4.9 msec. Their average latencies in several series were 1.3, 2.6, and 4.4 msec. Thus the average central reflex times of the first and second relayed waves were respectively 1.3 and 3.1 msec. corresponding to measured limiting frequencies of 340 and 200.

3. *Synaptic resonance.* Figure 5 begins with record A at a frequency of 160 per sec. There is a large direct wave with latent period 1.1 msec. It is followed by a very small relayed wave with latent period 2.3 msec. and a

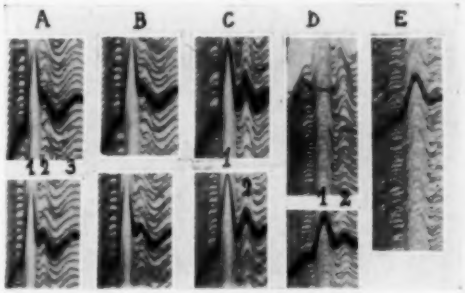

FIG. 5. Record A early and late portion of film, at 170 per sec., shows large direct wave immediately succeeded by just noticeable first relayed wave and broad second relayed wave. The first relayed wave is still small in B, at 198 per sec., but has greatly increased at stimulation rate 268 per sec. in record C. D at 310 per sec., and E at 360 per sec. Full description in text. To be read downwards.

second broad relayed wave at latent period 3.6 msec. The first relayed wave increases in size during stimulation in this and in the following record B at 198 per sec. In the next record C at stimulation rate 268 per sec. the first relayed wave is 4–5 times larger than in the beginning of the first record (A) and remains large for a considerable time during stimulation. Thus it has an optimum around this frequency range. The second relayed wave is still present in record D at 310 per sec. but on the verge of disappearing in record E at 360 which is the limiting frequency of this wave with an average central reflex time around 1 msec.

Certain relayed waves are often absent or questionable at slow rates of stimulation below 70 per sec. and turn up at higher rates again to disappear when the frequency increases. There are thus at times optimal rates of stimulation for certain relayed waves and in this sense true frequency resonance (2).

4. *Limiting frequency and central reflex time.* In correlating limiting frequency and central reflex time it is necessary to realize at the outset that such measurements cannot claim great accuracy. Several factors contribute to make precision unattainable. Latent periods are easier to measure with precision than limiting frequencies but partial overlapping of the waves in some cases and slightly increased latencies at faster rates of stimulation in others may make decision difficult. The average latent periods, leaving out the first 4–5 stimuli, have been used.

The limiting frequencies often interfere with the stimulus artifact and then may be difficult to determine. This is the main source of error especially at high frequencies. The relayed waves may be small or badly defined and we have no means of appraising strength of stimulation for a relayed wave, except in a very general manner. The limiting frequency in peripheral nerve, theoretically determined by the refractory period, is also determined by strength of stimulus and conduction distance (9) as well as by temporal dispersion caused by slowed conduction and increased latency (3). At higher frequencies it is necessary to use a brief period of stimulation and long intervals (9). These precautions have been observed. But the difficulties mentioned, in determining limiting frequency and others not particularly enumerated, all serve to emphasize that our curve, shown in Fig. 6, cannot do more than *indicate in a very general manner* the relationship between limiting frequency and central reflex time. There are also physiological factors causing variations when in other respects optimal conditions for measuring have prevailed.

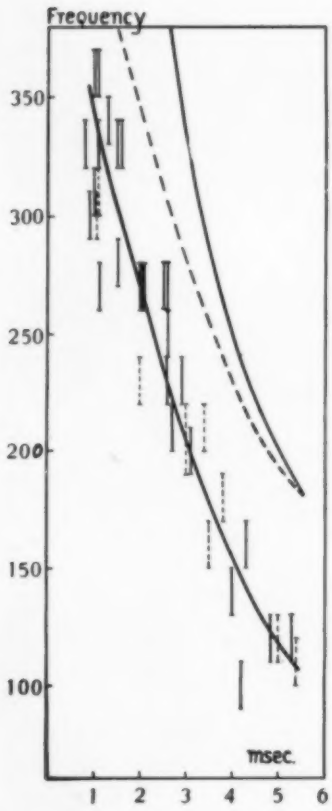

FIG. 6. *Curve on the left:* Limiting frequency of relayed waves (ordinates) plotted against central reflex times of same waves. *Curve on the right:* inverse values of central reflex times plotted against central reflex times. Curve *in broken lines:* curve to the left lifted upwards by a certain amount as explained in text.

In Fig. 6 data from crossed stimulation are marked by broken lines. It is at once seen that these data tend to be grouped in the lower half of the curve, whereas those taken from experiments with ipsilateral stimuli are grouped around the upper half of the curve. There are exceptions from this rule. But in general the central reflex times are longer for delayed waves elicited by crossed stimuli.

The upper curve is put in to illustrate the theoretical limiting frequency given by the inverse values of the central reflex times. The vertical distance between the theoretical and the experimental curve is, of course, also a function of the definition of the limiting frequency. The experimental curve could be pushed upwards by requiring only two shocks to be effective at a given frequency of stimulation, but practical reasons have necessitated a different choice of index, as pointed out above. But, if the experimental curve is lifted upwards to coincide with the theoretical curve at the lower end it can be seen that the two curves diverge for short latencies, meaning that the corresponding high frequencies possess a depressing effect augmented by stimulus repetition (cf. Fig. 1). This no

doubt is identical with the effect described by Graham and Lorente de Nó (13) and by them identified with the subnormality accompanying positive afterpotentials in peripheral nerve (10). As is well known from Gasser's work development of subnormality is greatly favored by rapid stimulation rates.

The shortest reflex times found in these experiments have been between 0.7 and 1.0 msec. Values below 1 msec. must refer to a single synapse (22, 17, 6). The maximal limiting frequencies have been about 360 per sec. Considering the rapid onset of subnormality with repetition, the absolute limiting frequency, defined as the capacity to pass only two volleys, would probably be a great deal higher. Alternation and intermittent conduction may also come in and complicate such determinations.

Discussion

The curves of Fig. 6 are interesting from two points of view, one eminently practical, the other theoretical. From the practical point of view it means that it may become possible to use curves of this type for analyzing the degree of complexity of the path between two points anywhere in the central nervous system, provided that the relation found is confirmed for other systems and that definite grouped discharges occur. So far we have only analyzed the relayed waves found in the dorsal columns (14) which have limiting frequencies falling around our curve. In this system there is also a direct wave which, of course, is necessary for establishing the form of the standard curve. But once this curve has been established for a given index in measuring limiting frequencies, the central reflex time can be located on it from measurements of limiting frequency alone, while the curve eliminates an important unknown factor, the datum provided by the direct wave. This can, of course, only be obtained for a relatively limited number of combinations of stimulating and recording electrodes.

When using the limiting frequency in this manner as an instrument for determining the central reflex time one may well ask whether it is possible to assume any fixed relationship between number of synapses and limiting frequency. Probably there exists some general relationship and probably number of relays is a decisive datum in determining a given point on the curve. But the dispersion of the relayed waves and the great variations indicate that other factors also play a rôle. The "delay path" (Forbes) responsible for a central reflex time of 5 msec. may, for instance, be determined by a minimal number of five synapses but delayed conduction owing to decreased fibre diameter and lengthened conduction distance are factors which hardly can be neglected and suggest some caution in interpreting the data. Still, we are inclined to hold that a *minimal* average number of 1 synapse per msec. is a reasonable assumption. The number may be greater but hardly less. It should also be realized that the first synapse occupies a favorable position compared with synapses placed later in the path on account of the more perfect synchronization of the stimuli for this synapse. This may be the reason why, as we have found (unpublished data), the

motor end plate with a synaptic delay of 0.5 msec. (7) has much higher limiting frequencies than those found in the central nervous system, though in the latter the synaptic delay is of the same order (18, 19). A highly dispersed discharge as such would indicate complex pathways.

From the theoretical point of view the interest centers around the relation of these results to possible mechanisms of inhibition. Gasser's view that subnormality may be one such mechanism is borne out by the frequency effect upon the relayed waves activated by high frequencies capable of piling up subnormality very quickly. The frequencies concerned are within the physiological limits of the sense organs. For this type of inhibition McCouch, Hughes and Stewart (21) very properly suggest the use of Dusser de Barenne's term "extinction." But a new possibility is introduced by the fact that the delayed discharge caused by every single repeated stimulus is cut down from the "tail" end by relatively moderate frequencies. This suggests that long-synapse paths are particularly inhibitable merely on account of the repetitive nature of the physiological stimulus. It also suggests that inasmuch as the natural stimuli for some reason or other set up high frequencies the immediate effects are transmitted with preference into the synaptic channels of least resistance, *i.e.*, those with brief central reflex times. It is clear therefore that a frequency variation as such apart from how it may facilitate or block certain paths is a mechanism of significance in determining the central distribution of an effect entering a path containing synapses.

Late facilitation effects such as the recruitment and the synaptic resonance, described above, emphasize aspects of stimulus repetition which do not come out in the curve plotted as Fig. 6. Considering that in the superior cervical ganglion there is a late facilitation which has not required internuncial bombardment (4, 5); that this phase coincides with a slow negative potential change, and, further, that recruitment in peripheral nerve is favored by factors favoring the negative afterpotential (12, 8), it is probable that slow potential changes may serve to modify the central processes also in a positive direction. Synaptic resonance, demonstrated so clearly in the transient reflex discovered by Bernhard and Skoglund (2), is an interesting consequences of what different balancing influences may lead to.

Summary

When trains of electric stimuli are led through needle electrodes to the spinal cord certain wave patterns are set up which have been recorded from the sciatic nerve (decerebrate cats). These consist of a direct wave caused by stimulation of the ventral horn cells and by a number of relayed waves following after different latent periods.

By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained.

The direct waves follow frequencies of stimulation above 850 per sec.

The relayed waves follow frequencies which decrease with increasing central reflex times. A curve illustrating limiting frequency as a function of central reflex time is given in Fig. 6.

Some relayed waves gradually recruit neurones and thereby increase in size, others have definite frequency optima and thus demonstrate the existence of synaptic resonance.

The findings are discussed in relation to excitation and inhibition and also with a view to suggesting use of the limiting frequency as a method of studying the central paths.

Variations in frequency as such can serve as a means of distributing into special channels effects entering a central path containing synapses.

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THE CENTRAL EXCITATORY STATE ASSOCIATED WITH POSTURAL ABNORMALITIES

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INTRODUCTION

STRIATED muscle frequently exhibits varying degrees of rigidity in the absence of either voluntary contraction or organic neuromuscular disease. In the clinical literature this is usually called "spasm," yet there have been few analytical studies of this phenomenon. Jacobson (10) described what he termed "neuromuscular hypertonus," attributing the contraction in muscle to mental stresses.

Buchthal and Clemmeson (4) reported 46 cases in which electromyograms were made of "palpable muscle affections." They found "rest activity" in eight instances, and in a number of others, particularly where movement was painful, activity disappeared when the patient was arranged in a comfortable position and his attention diverted. In the balance, no activity was noted.

In an earlier paper, one of us (J.S.D.) described action currents in human muscle which, when palpated, was abnormally tender and resistant to pressure deformation. The muscles examined were spinal extensors and the subjects were at rest in the prone position with the head in the midline. The activity was not constant in degree; at times it did not appear for a considerable period after the electrodes were placed (1–45 min.), and at others it came in and dropped out without apparent cause. The subjects, except one, had no organic disease. Each, however, had a postural abnormality. Because this activity seemed to be a definite departure from normal, and because the afferent limbs of the reflex arc were not observed objectively and hence must be considered in general terms, the muscle activity seen in these studies was termed a "lesion reflex," and the areas called "lesion areas."

Since it had been noted in most lesion areas that activity could be induced by merely touching the electrodes, not a normal finding in our experience or in that of others[1] (4), it was apparent that the needle stimulus summed with a subliminal C. E. S. (6), to cause reflex action.

Wright (18) cites the work of Mackenzie who postulated that muscle contraction, persistent or evoked by palpation, might be an exaggerated

[1] Buchthal and Clemmeson, in commenting on the temporary effect of needle irritation state "Immediately after pricking with a needle electrode, there can appear in some muscle fibers a short (<1 min.) irritation with corresponding muscle discharge (pricking potentials). We have made the same observation. When the electrodes are in normal muscle, the skin about them may be moved to a considerable degree without initiating action potentials."

reflex response due to an area of "irritable focus" probably located in the posterior horn and created by an abnormal stream of impulses from certain pathological viscera or serous membranes. It seems probable that a pathological focus in structures other than viscera or serous membranes might create an area of "irritable focus" and thus might account for spontaneous or easily induced reflex activity.

Because of the importance of such activity, particularly in bio-economy and fatigue, further observations are reported.

METHODS

Dual channel, balanced (Offner Type 140) amplifiers drove a cathode ray oscilloscope, a loud speaker, or Westinghouse bifilar oscillographs for recording on bromide paper. In most of the experiments concentric needle electrodes (22 gauge) were used. Two needle electrodes and a needle and a skin pad electrode were used occasionally.

The subject was placed in the prone, sitting or standing position. When the subject was prone, the face was placed in a padded slot in the table; when sitting or standing the subject assumed a comfortable, "balanced" position except in a few experiments when records were made with the subject either standing on one foot or the other, or standing in a position of full forward flexion. Areas of lesion and normal control areas were selected by palpation and electrodes inserted. Electrodes and skin were treated with 70 per cent alcohol and the skin was anesthetized by intradermal injection of 1–2 cc. of 2 per cent procaine hydrochloride. The differential criteria between lesion and normal areas have been described elsewhere (5). In this series lesion areas have been classified arbitrarily as "major" and "minor." Major lesions are characterized by considerable abnormal firmness, rigidity and tenderness. In most instances the subject complained of having had discomfort in the area. Minor lesions are less rigid and tender and the subjects were usually unaware of the abnormality. Normal areas are soft, resilient and not tender.

When spontaneous activity was not present in a lesion area, stimuli were applied either halfway between the electrodes in the lesion and normal areas or equidistant from each. These stimuli consisted of slight movement of the electrodes, pin pricks or pin scratches, too light to cause mass contraction in the area,[2] cubes of ice with a surface area of approximately 2.5cm.^{2}, and pressures of 1000–1500 mm. of Hg delivered through a round cork which had a surface area of 175 mm.^{2} Forced inspiration and changes in position and weight bearing were also used.

Most experiments occupied approximately one hour. During this period constant observations of the lesion and normal areas were made with the oscilloscope and loud speaker. Photographic records were made at various intervals with the subject relaxed and in the quiet period between exhalation and inhalation (most of the muscles observed were accessory muscles of respiration.) We have found, as did Lindsley (12), that relaxation of normal muscle is not difficult to attain. Because voluntary contraction and involuntary tension show activity similar to reflex contraction, all records were taken with one electrode in a lesion area and another in a nearby normal control area. It was assumed that absence of activity in the control area was adequate to show an absence of either voluntary contraction or involuntary tension. While it is acknowledged that a trained subject might isolate voluntary activity to the lesion area, leaving the control quiet, these subjects were not trained, and care was taken to avoid letting them know what channel was feeding the speaker.

The areas examined included the spinal extensors, the glutei, the tibialis anterior and the extensor digitorum longus.

SUBJECTS

Thirty-two records were taken on 16 men and one woman, including students and instructors, all between the ages of 21 and 36. Fifteen presented some degree of postural

[2] In a control experiment with both electrodes in normal areas, pin pricks sufficiently sharp to cause bleeding did not initiate activity. These were heavier than those used in other experiments.

abnormality. Six had been subjects in the previous series; all were free from gross disease and were carrying full work. Only one had been incapacitated from injury in the past five years. He suffered a fractured elbow one and one half years ago and made an uneventful recovery.

Although these subjects are ordinarily classified as "normal" or "healthy," those with major lesions have a history of discomfort, in varying degrees, in the lesion area. Likewise, these individuals report fatigue at physical effort. No measurements of this factor have been made. Two subjects were excellent physical specimens and provided good normal control observations. Except for a minor pollen sensitization in one, both have been unusually free from minor illness and have good capacity for work without fatigue.

Results

Spontaneous activity. This was considered to be present when one or more motor units were active in the absence of voluntary contraction or

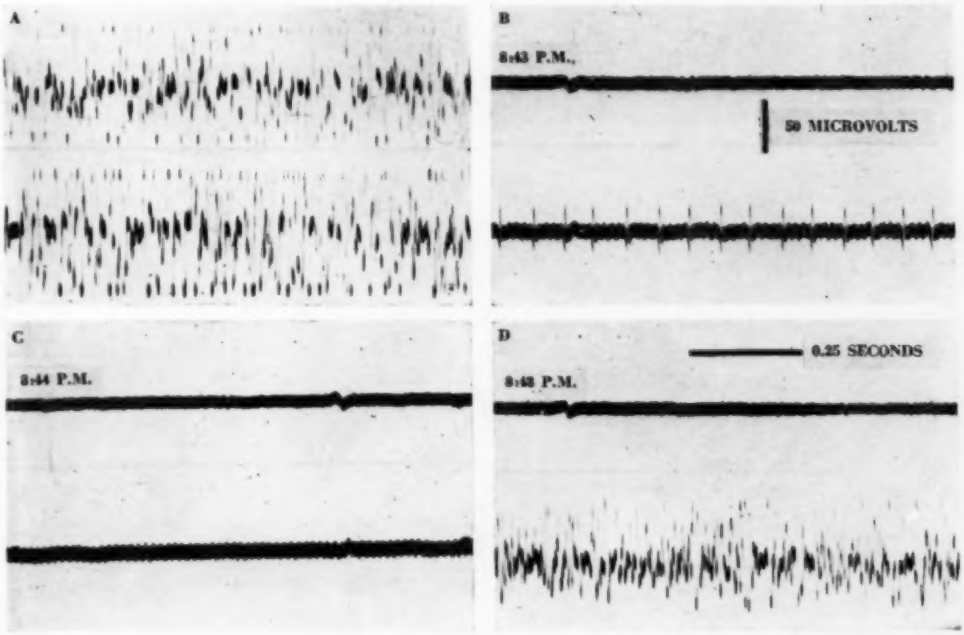

Fig. 1. Varying degrees of activity in lesion area. Upper tracing—concentric electrode in normal area about 3 cm. to left of sixth thoracic spine. Lower tracing—concentric electrode in lesion area about 3 cm. to right of sixth spinous process. (A) voluntary contraction to show that both electrodes were in muscle. (B) single unit firing in lesion area. (C) both areas quiet. (D) many units active spontaneously in lesion area.

involuntary tension after the flurry of activity induced by electrode insertion subsided. This activity was seen in twenty out of twenty-five observations of areas of major lesion. It waxed and waned in degree, varying from quiet periods to single unit activity to mass contraction (Fig. 1). No sharp pattern for the rise and fall of activity could be detected. Factors which usually increase the frequency and number of units firing, without affecting the nearby control area, are inhalation, painful stimuli, apprehension and fatigue from being in one position. Factors which usually decrease the fre-

quency and volume of activity are a deep breath taken slowly, a slight change of position and mental concentration. However, a given factor might cause a decrease in activity at one time and an increase at another. Two examples can be cited.

When a single unit is active in a lesion area in the thoracic extensors, the frequency of firing often increases with inspiration (Fig. 2). In two experiments attempts were made to get photographic records of this phenomenon. One subject became tense while receiving instructions and many additional units became active. In the other, while instructions were given, the active unit faded and was quiet for six minutes when other units came in spontaneously. In the latter experiment the quiet control area fed the speaker while the activity in the lesion area was switched to the oscilloscope,

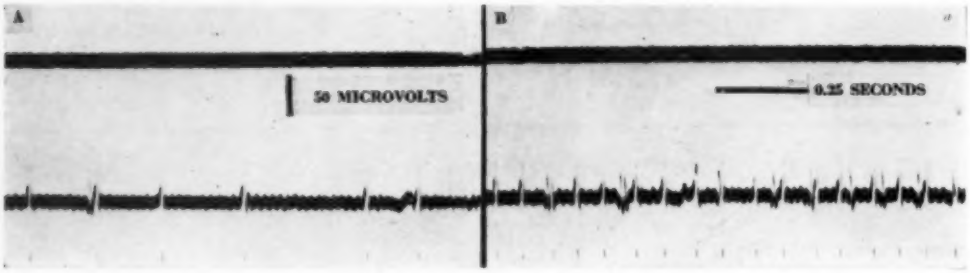

FIG. 2. Varying frequency of single unit during inhalation and rest periods of respiratory cycle. Upper tracing—concentric electrode in normal area about 3 cm. to right of fourth thoracic spine. Lower tracing—concentric electrode in lesion area 3 cm. to right of sixth thoracic spine. (A) subject inhaling. (B) rest period between active exhalation and inhalation.

out of the vision of the subject; he was unaware of the progress of the experiment.

Often there was rotation of spontaneous activity, different units coming in or dropping out without apparent cause. In one experiment (Fig. 3) a single unit fired during the rest period before inhalation. It persisted through inspiration and part of exhalation but faded during the ensuing rest period. Seven seconds after the beginning of the first, the next inhalation started. Units different from the original single one became active. Meanwhile, the control area remained quiet. Four minutes later the original single unit started to fire spontaneously.

Absence of spontaneous activity. In 5 records from areas of major lesion there was no spontaneous activity found in observations which extend over 65, 95, 82, 49 and 117 min.

In 4 experiments on 4 subjects there was no spontaneous activity in minor lesions. In two of these during other experiments, the muscle rigidity was more marked and spontaneous activity was shown.

Induced activity. We often noted that a slight movement of the skin around an electrode in the lesion area was followed by motor unit activity while the control area, similarly treated, remained quiet. This suggested a

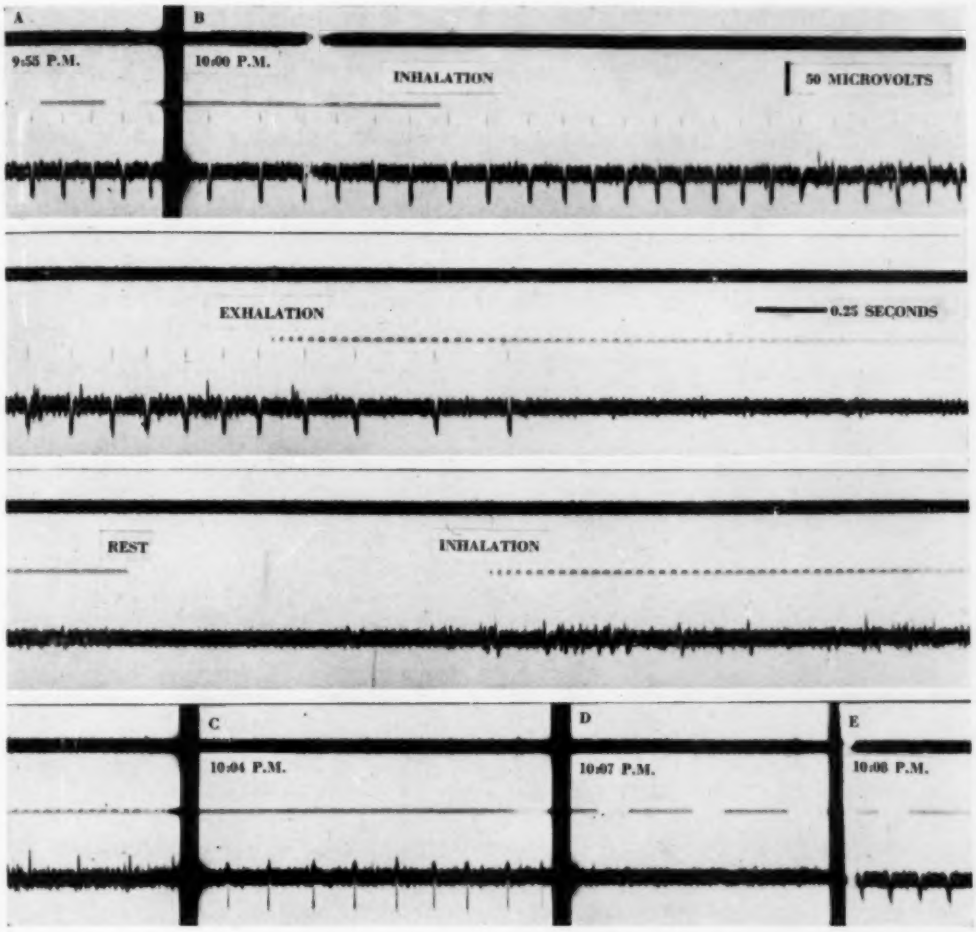

FIG. 3. The effect of respiration on reflex activity of the spinal extensors at the level of the fifth thoracic vertebra; concentric electrodes in normal (upper tracing) and lesion (lower tracing) areas. (A) single unit firing. (B) phases of respiratory cycle as marked. (C) spontaneous renewal of activity in lesion area. (D) extinction of unit shown in (C) after movement by subject. (E) spontaneous renewal of activity lesion area, apparently by the same unit shown in (A). Time marker (0.25 sec.) shown in (A) and (D) used in (B) to indicate change in phase of respiration.

means of differentiating, by means other than palpation, a normal area from a lesion area in which no spontaneous activity was occurring. Accordingly, when neither normal nor lesion areas showed activity after the electrodes were placed, the area between the electrodes (generally the skin over the spinous processes) was stimulated by pricking or scratching with a needle, by direct pressure or by the application of a cube of ice. Occasionally such stimulation evoked no activity but in general motor unit activity in the lesion area began soon after its application. Figure 4 shows a record of such activity induced by scratching the skin.

In the present series of experiments, stimuli as described above were applied fifty-five times. Activity in the lesion area was evoked forty times (72.7 per cent) and in control areas only five times (0.9 per cent). Activity thus induced in control areas was always transitory, while in the lesion areas it frequently persisted over relatively long periods (up to 10 min.).

In addition, two subjects who presented no obvious symptoms of neuromuscular lesions were used as controls. Out of ten applications of various stimuli, only once was there evidence of even minor activity.

Subjects sitting, standing erect, standing flexed, standing on one foot. It is interesting to note that in the spinal extensors in a few experiments the activity or lack of activity characteristic of the normal and lesion areas does not vary greatly when the subject is in a weight bearing position,

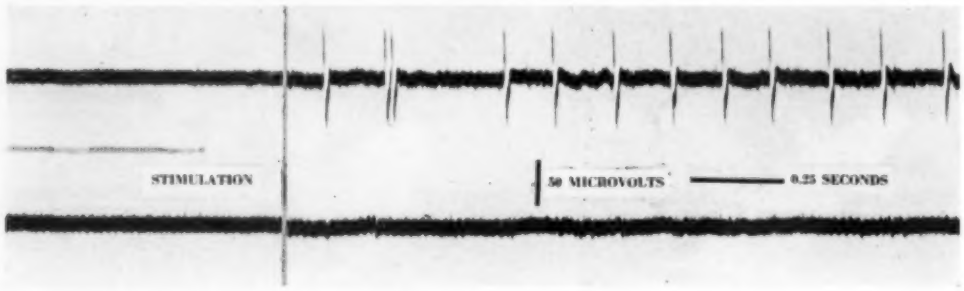

FIG. 4. Motor unit activity induced in a lesion area by scratching the skin between the electrodes. Upper tracing, lesion area; lower tracing, normal area. Time from application of the stimulus (indicated by cessation of time marker) to beginning of activity, 4.5 sec.

provided he is able to stand in an easy position. Further observations are being made and will be reported at a later date.

Activity in control area. There is no sharp line of demarcation between a lesion and a normal area, the former shading into the latter. At times, to get a nearby control it was necessary to put the electrode in the fringe of the lesion. If spontaneous or easily induced activity was seen, the needle was moved to a quiet area. If the activity was occasional and if the area was quiet while activity in the lesion area was being observed, it was considered a valid control. Likewise we sometimes erred in differentiating normal from lesion areas, probably due to the depth of tissue.

Non-rhythmic action potentials. Smith (16) reported irregular frequencies and stated "at threshold a unit may discharge in quite a random way without establishing any rhythm at all." We have seen this in several experiments. In one experiment a photographic record which ran 27 sec. showed one unit which fired twice and one which fired four times. This type of activity was observed throughout much of the experiment (77 min.).

From time to time the action potentials appearing on the photographic record present unusual patterns. In Fig. 5, (A) shows double spikes which have appeared in several records. The latency of these double spikes is

approximately 8.0 σ. In the same figure (B) and (C) show bursts of activity which in (C) occur with some regularity. These bursts are composed of large single discharges followed by a shower of lesser ones. In the same record can be seen a rhythmic single unit which continued to fire after the bursts had ceased spontaneously (D).

Activity in muscles with a common nerve supply. In most experiments it was difficult to identify accurately muscles under examination. In one, however, the tibialis anterior was the lesion area, the homolateral extensor

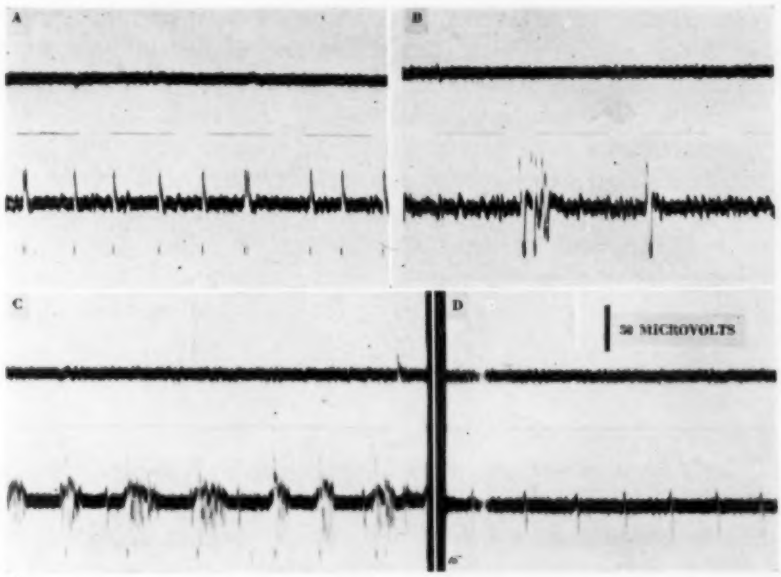

FIG. 5. Unusual types of action potentials. (A) double spikes; (B) irregular spikes. (C) small single spike combined with bursts. (D) same single unit firing alone 2 min. later.

digitorum longus being the control. There was a sharp difference in rigidity and tenderness; the latter was normal and the former quite firm and tender. There was alternating rhythmic and random activity in the lesion with the control quiet for 77 min. Obviously, although the deep peroneal nerve supplies both muscles, they receive motor fibers from different areas in the anterior horn.

The results in hand as regards spontaneous muscle activity, the recruitment and the frequency variation of such activity, as well as its evocation during quiet periods, confirm the observations which were reported in an earlier paper (5).

DISCUSSION

Since the rhythmic action potentials of single motor unit activity were originally shown by stimulating single nerve fibers (1, 2), it has been established that such activity, when it occurs *in vivo*, is the result of reflex action.

The motor unit is the "final common path" (6, 14); its activity is the result of afferents reaching the spinal cord at a given level, hence it may be used as an indirect indicator to ascertain the level of any bombardment of afferent impulses.

Jacobson (10), Lindsley (12), Smith (15), Seyffarth (13), Hoefer and Putnam (7), and Buchthal and Clemmesen (4) have reported an absence of muscle action currents in the normal resting muscle. This is supported by our observations. Hence, we have concluded that the presence of spontaneous or induced activity in such muscles is abnormal. Since this activity occurs commonly in muscles which are rigid and in areas of postural abnormality, it strongly suggests that these are related and interdependent phenomena. Although the rigid muscles in the lesion areas resemble, in texture, muscles in voluntary contraction, the spontaneous or induced activity in the lesion area is usually less than that of voluntary contraction.

In a single clinical experiment on a case of suspected tetanus, Watkins (17) used the effect of an external stimulus on the action potentials of the affected muscles to confirm the diagnosis and to follow the process of recovery. The electromyograms showed that the sound of a whistle greatly increased the action potentials of the affected muscles, even though they were ostensibly relaxed. This indicated that the state of hyperirritability characteristic of tetanus existed, and that the central nervous system was involved.

The evocation of additional activity by a stimulus which, in the normal would be ineffective, is explained by Sherrington's[3] (14) concept of a motoneuron pool in which there is an enduring subliminal central excitatory state (C. E. S.) created by subthreshold stimuli.

Apparently, the phenomenon of spatial summation operated to cause massive blocks of motoneurons to fire. Although Watkins' study was made on tetanus, and our own on rigid muscle in postural abnormalities, our observations of action potentials resulting from a minor stimulus parallel his.

In this phase of the discussion the size or cause of the enduring C. E. S. is less important than the fact that such states have been observed in supposedly healthy individuals.

Steindler (16) regards vertebral joints as being capable of absorbing ordinary stresses and strains in the distributive mechanism of the ligamentous apparatus; he points out that static disalignments, certain anatomic types and anatomic abnormalities disrupt this mechanism with resultant symptomatology. This coincides with our observations that the rigid muscles occurring in postural abnormalities show electromyographic characteristics different from muscles which have normal texture.

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[3] The discussions of the enduring C. E. S. indicate that it lasts from ten to twenty σ. We have been unable to find data on the time it may persist with repeated stimuli which are subthreshold for either direct activity or summation. Our observations indicate that the subliminal C. E. S. may persist for indefinite periods, weeks at least. In one subject activity was induced in the lesion area in three experiments in an eight-week period.

However, the fluctuations in activity and particularly the change of threshold for various stimuli imply more fundamental changes than the merely uncomfortable orthopedic disturbances. In but one aspect, a study of the difference in metabolism between normal resting muscle without electrical activity and the spontaneously active or hyperirritable muscle should lead to knowledge which will provide a better understanding of the mechanism of fatigue.

Fluctuations in activity in the lesion area might result from changes in the environment of the afferent end organs, from extraneous factors affecting reflex activity in a given cord segment or, less probably, from changes in the motor unit. Further speculation at this time would be unfruitful, as direct studies of afferent activity have not been made and the limited knowledge of C. E. S. and C. I. S. precludes the possibility of drawing direct parallels.

The significance of the various types of arhythmic potentials which appear at times is very doubtful and it is hoped that more evidence can be accumulated. It is very interesting to note, however, that the double spikes shown in Fig. 5 are seemingly identical with those shown in a previous report (5). These spikes might be explained on the basis of: (i) double discharges of a single motor unit; (ii) other units firing almost synchronously; (iii) asynchronous discharge of fiber groups in a single unit.

Summary

1. Rigid muscles (lesion areas) associated with postural abnormalities commonly show spontaneous action potentials.

2. When such spontaneous activity is absent, it can be induced by suitable stimuli; this is a useful criterion for detecting lesion areas.

3. Induced reflex activity is probably dependent upon the presence of an enduring C. E. S. plus an accessory subliminal stimulus.

4. Various unusual types of action potentials occur; these are of doubtful origin.

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THE FUNCTION OF COMPONENTS OF THE RESPIRATORY COMPLEX[1]

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TRANSECTION of the brain stem has been widely used in the past as a method of delimiting the minimum respiratory neural complement necessary to support life. From such studies it appears that the caudal part of the bulb below the level of entrance of the eighth cranial nerves, the spinal motor pathways and certain reflex connections, constitute the essential parts of this mechanism (9). More recently the confines of the bulbar respiratory center have been more exactly mapped by localized electrical and chemical stimulation and by the recording of spontaneous action potentials in phase with respiration (4, 7, 8, 12, 24). These investigations confirm and add a measure of refinement to the localization established by gross transection.

However, Marckwald (19) and later Lumsden (18), Stella (29) and Pitts, Magoun and Ranson (26) described a more rostral division of the central respiratory mechanism subserving, along with vagal inhibitory afferents, the important function of contributing rhythmicity to respiration. This rather ill-defined rostral level was called by Lumsden the pneumotaxic center. The bulbar respiratory center, freed from inhibitory mechanisms within the upper brain stem and released from those stretch afferents within the vagus nerves which inhibit inspiration, passes into a state of maintained tonic activity resulting in inspiratory cramp (Marckwald) or apneusis (Lumsden).[3] Stella (30), especially, has strengthened the view that the inspiratory cramp results from unrestricted activity of the inspiratory center by showing that the magnitude of the cramp, *i.e.*, the depth of the maintained inspiration, is directly related to the carbon dioxide content of the inspired air.

Activities of individual inspiratory motor neurones have been studied extensively by a number of investigators in animals in which the entire neuraxis was intact (2, 6, 11). The behavior of these motor neurones under a variety of experimental circumstances is well known. It was felt that observation of the pattern and character of this neural discharge could be

[1] Aided by a grant from the Penrose Fund of the American Philosophical Society.

[2] Lucius N. Littauer Fellow of Neurophysiology.

[3] It is our impression that the term *pneumotaxic center* has given the false impression of a compact aggregate of neurons anatomically and functionally homogeneous, all of which must be eliminated to produce inspiratory cramp. More in line with experimental fact, is the view that there are a number of reflex connections at various levels within the upper and middle brain stem, the elimination of a sufficient proportion of which leads to inspiratory cramp. It is for this reason that we have returned to the less specific term, *brain stem inhibitory mechanisms*. Use of the term *apneusis* has unfortunately been construed as meaning the acceptance of Lumsden's terminology and views in their entirety. We therefore feel justified in returning to Marckwald's original descriptive term, *inspiratory cramp*.

used to assess, more accurately than observation of respiratory records alone, the effects of transection of the brain stem of the cat at various levels and of section of the vagus nerves. Transection methods are subject to criticism because of the associated shock and trauma and it is our belief that these factors can be evaluated better by a study of the neural discharge than of the respiratory records.

METHODS

The experimental methods employed for recording action potentials of phrenic neurones and respiratory volume of the cat are essentially those described by Pitts (23). The respiratory recording system was arranged so that the soda-lime absorption tube could be quickly shunted out of the system to test the animal's response to carbon dioxide. Transection of the brain stem was performed under ether anaesthesia. In the ensuing two hours or more, required in the preparation of single phrenic neurones, the greater part of the ether was eliminated from the body. Hence anaesthesia is not a complicating factor in these experiments.

Studies have been made in animals with the brain stem transected at either of two general levels. The more rostral of the two levels was the usual intercollicular section of Sherrington. The more caudal level passed through the acoustic tubercles dorsally and either the caudal pons or trapezoid body ventrally. In all instances the brain was removed at the end of the experiment and examined grossly for level and completeness of transection. Mortality was high in the animals transected at the caudal level despite prolonged artificial respiration. Of those animals which survived the low transection, some, on section of the vagus nerves showed slow prolonged rhythmic inspirations at a frequency of 2–4 per min. (19), sufficient to maintain life for a considerable period of time. The majority, however, showed true inspiratory cramp, *i.e.*, when the vagus nerves were cut, inspiration occurred at once and was maintained until the animal died in from four to eight minutes. Only results obtained on the latter type of preparation are considered here.[4]

Criteria of Normal Phrenic Activity

In a study of the pattern of activity of respiratory motor units, Gesell Magee and Bricker (13) have pointed out that phrenic units characteristically show a slowly augmenting repetitive response during inspiration which ceases abruptly with the onset of expiration. Examples of this slowly augmenting and rapidly decreasing response may be seen in Fig. 1 to 5. Almost all phrenic units which we have observed, either with or without anaesthesia, show this type of response so long as respiration continues rhythmic and regular.

Depth of respiration has been shown by Adrian and Bronk (2), Bronk and Ferguson (6) and Gesell, Atkinson and Brown (11) to be regulated in two ways: (i), by the frequency of discharge of the several motor units and (ii), by the number of motor units active. These observations have been repeatedly confirmed in our experiments on phrenic motor neurones (Fig. 4 and 5). Together with observations of the pattern of activity, they constitute criteria of normality by which we may assess the effects of transection of the brain stem and section of the vagus nerves.

[4] In a recent preliminary report Nicholson and Hong (20) have described a somewhat different respiratory response of the dog to combined section of the pons and vagi. A decision as to whether these results indicate a species difference between cat and dog or are dependent on other factors must await a complete report of their results.

Mechanisms Determining the Pattern of Inspiratory Activity

Eupneic respiration is ordinarily an active process only with respect to inspiration, expiration resulting from passive recoil of elastic chest structures. Observation of activity of phrenic motor units provides, therefore, an adequate picture of the neural processes of eupneic breathing. In Fig. 1, the top record shows the activity of two such phrenic motor units, distinguishable by differences in magnitude of spike potential. Both units show a slowly augmenting frequency of response during inspiration, which suffers sudden decrement with the onset of expiration. The records of Fig. 1 were obtained from an intercollicular decerebrate animal in which both

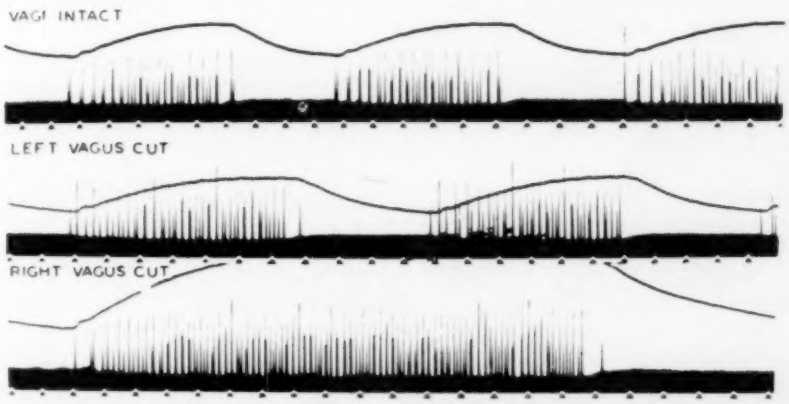

Fig. 1. The effect of section of the vagus nerves on the discharge of impulses by two phrenic neurones of a high decerebrate (intercollicular) cat. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{5}$ sec.

the brain stem and vagus mechanisms which inhibit inspiration were intact. Section of first the left and then the right vagus was followed by the well known prolongation of inspiratory activity and slowing of the rate of respiration. The loss of those stretch afferents in the vagus which normally cut short inspiration (1, 15) is undoubtedly the major causal factor in the prolonged inspiratory activity seen in the lowermost record.[5] The general pattern of activity on the other hand, is not significantly altered. Inhibition of inspiration after bilateral vagotomy is eventually brought about by activity of those brain stem inhibitory mechanisms which remain intact in the intercollicular decerebrate animal.

[5] In confirmation of the results of Larrabee and Knowlton (16) we have seen no evidence of activity of stretch afferents in the vagi which excite inspiration during normal eupneic respiration (14). The rate of augmentation of frequency of phrenic neurone discharge is essentially the same with vagi intact as with vagi cut. The peak frequency of response is, as a matter of fact, usually higher after vagal section, and recruitment of additional neurons often occurs. Both results are probably dependent in part upon higher carbon dioxide content of the blood due to less effective ventilation.

The low decerebrate animal, in which these mechanisms have been eliminated, breathes in an essentially normal manner so long as the vagi are intact (upper record of Fig. 2). The characteristic slowly augmenting and rapidly decreasing frequency of phrenic neurone discharge is evident. Section of the left vagus prolonged inspiratory activity and slowed the rate of respiration. Bilateral vagal section led to continuous repetitive discharge of this neurone, and to the recruitment of another, characterized by a spike potential of greater magnitude. The picture is one of an even more complete removal of inhibitory influences acting on the respiratory center than that seen in the lower record of Fig. 1.

A partial restoration of conditions essential to rhythmic respiration may be effected by intermittent stimulation of the vagus with low intensity, high frequency stimuli (26). A study of the behavior of phrenic neurones under these conditions reveals how very closely the activity pattern of this

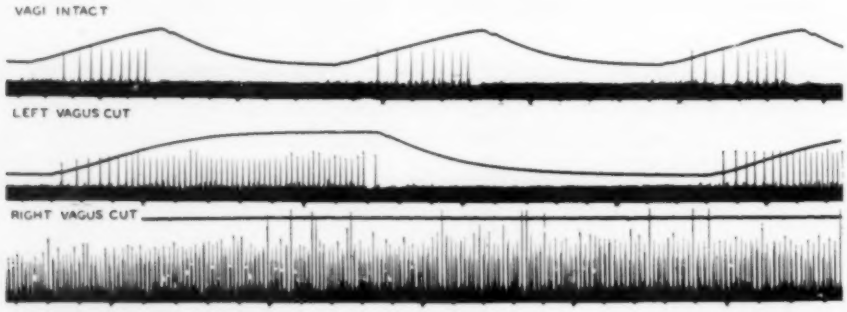

FIG. 2. The effect of section of the vagus nerves on the discharge of impulses by two phrenic neurones of a low decerebrate (low pontine) cat. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{4}$ sec.

artificially induced rhythmic respiration approximates the normal. Figure 3A is a continuation of Fig. 2 after bilateral vagus section. In Fig. 3B and C, repetitive central stimulation of one vagus, for the time indicated between the arrows, restored respiration to something approximating that of unilateral vagal section (Fig. 2, middle record). A slow augmentation of frequency of phrenic discharge and a relatively rapid decrement is evident. Higher intensities of vagal stimulation, though leading to more rapid inhibition, invariably showed evidence of brief excitation prior to inhibition (possibly the excitatory vagal afferents of Gesell and Moyer, 14). In general features, however, the respiratory pattern of record C approximates the normal quite well.

An additional feature of Fig. 3 is worthy of note. Inhibition resulting from a relatively short (about 0.8 sec.) burst of stimuli to the vagus, persists for some time (1.4 to 3.0 sec.) before activity recommences. Furthermore, the neurone of greater spike potential (Fig. 2, bottom record, and 3A and B, first part) is maintained inactive with brief repetitive central stimulation of the vagus at the intervals shown in Fig. 3B and C. It seems

possible that those central inhibitory changes which lead to cessation of inspiration during stimulation, persist in diminishing degree for an interval after stimulation ceases. The augmenting frequency of phrenic neurone discharge at the onset of the next inspiration might thus be a function, in part, of the progressive decay of this inhibition.

If one compares the pattern of inspiratory discharge determined by activity of the brain stem inhibitory system (Fig. 1, bottom record) with that determined by vagal afferents of an inhibitory nature (Fig. 2, top record, and 3C) the fundamental similarity is evident. In normal eupneic respiration the vagal inhibitory mechanisms are probably the more domi-

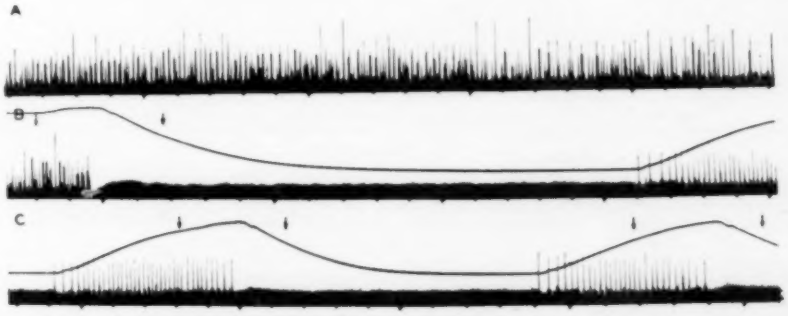

FIG. 3. The effect of stimulation of the central and of the right vagus nerve of a bilaterally vagotomized low decerebrate animal on the discharge of impulses by two phrenic neurones. Record A, a continuation of the lower record of Fig. 2; B and C, stimulation of the vagus during the interval between the pairs of arrows with shocks of near threshold intensity and a frequency of 100 per sec. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{4}$ sec.

nant ones, since section of the vagi leads to a profound slowing of respiration (at least initially). It is possible, however, that there is some mutual reenforcement of the activities of the brain stem and vagal inhibitory systems.

MECHANISMS DETERMINING DISCHARGE FREQUENCY AND RECRUITMENT

The ultimate stimulus to the respiratory center is probably a chemical one. Carbon dioxide, within limits, excites the respiratory center to a degree proportional to its tension in the blood stream. Translated into terms of normal phrenic neurone discharge, with increase of chemical stimulus the frequency of discharge of individual neurones is increased, inactive neurones are recruited, activity of certain neurones begins earlier in the inspiratory cycle and in addition the rate of respiration is increased. The operation of these factors is evident in the records of Fig. 4 (A to D), taken from a small slip of the phrenic nerve of an intercollicular decerebrate animal. The soda-lime absorption tube was shunted from the system and a gradual

accumulation of carbon dioxide was effected by rebreathing. The increase in frequency of discharge is evident in the response of the neurone of greater spike potential; recruitment of another neurone of lesser spike potential is evident in records C and D. As a consequence of the operation of such mechanisms, inspiration increased in depth. Rate of respiration also increased significantly from A to D.

Repetition of rebreathing in the same preparation after bilateral vagal section yielded results shown in Fig. 4 (E to H). These records were taken at time intervals approximately equal to those of Fig. 4 (A to D). Respiratory rate was slowed markedly as a result of vagotomy and showed no increase during rebreathing. However, frequency of impulse discharge in-

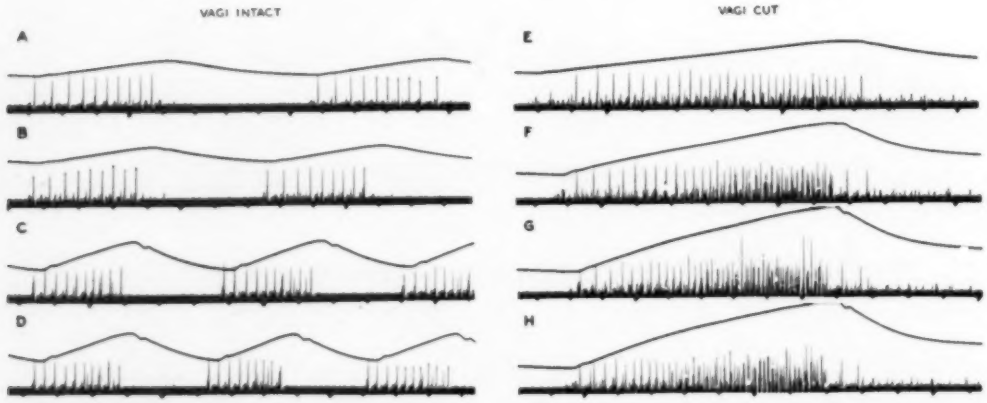

FIG. 4. A comparison of the effects of increasing tensions of carbon dioxide in the inspired air on the discharge of impulses by a few phrenic neurones before and after section of the vagus nerves (high decerebrate preparation). A to D and E to H, approximately equivalent increases in carbon dioxide before and after vagal section. Upstroke of the respiratory record indications inspiration. Time: $\frac{1}{5}$ sec.

creased much as it did with vagi intact and recruitment of additional neurones is even more evident. As a consequence depth of inspiration increased during rebreathing while rate remained essentially constant. Similar results have been obtained in several additional experiments.

Scott (28) and Larrabee (17) observed that the increase in rate in response to carbon dioxide inhalation is largely dependent on the integrity of vagal reflexes. Schmidt (27), however, has observed instances of rate increase after vagotomy, and observation of results obtained on a series of animals in our student laboratory indicate that in some a substantial rate increase may occur on rebreathing after bilateral vagal section. However, the mechanisms responsible for increase in impulse frequency and recruitment (the chief factors in increasing depth of respiration) are certainly resident in the brain stem below the level of intercollicular decerebration and are independent of the vagus nerves.

Repetition of these experiments in low decerebrate animals, *i.e.*, with

brain stem inhibitory mechanisms excluded, yield results, an example of which is presented in Fig. 5. This animal breathed rather slowly and deeply as shown in record A. Rebreathing, however, increased the respiratory rate some 30 per cent from A to C (measured on longer strips of record). In addition, the neurone of greater spike potential showed an increase in frequency of discharge, and in record C, another neurone of lesser spike potential was recruited. The response of the low decerebrate animal is in all fundamental respects similar to that of the intercollicular decerebrate animal as long as the vagi are intact.

Section of the vagi of the low decerebrate animal resulted in maintained phrenic discharge as shown in Fig. 5 (D to F). Record D followed immedi-

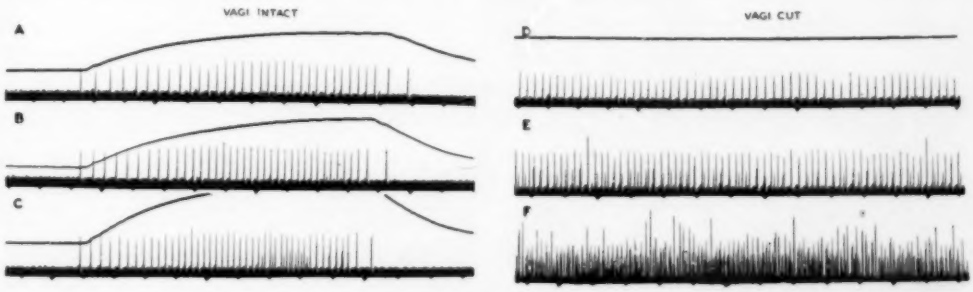

FIG. 5. A comparison of the effects of increasing tensions of carbon dioxide in the inspired air on the discharge of impulses by a few phrenic neurones before and after section of the vagus nerves (low decerebrate preparation). A to C, increasing carbon dioxide before vagal section; D to F, increasing carbon dioxide after vagal section. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{5}$ sec.

ately upon vagal section, E and F, at intervals thereafter. Shortly after record F was taken the discharge became irregular and the animal died. Records D to F are comparable in a sense to the rebreathing records of Fig. 4 and 5 (A to C). Carbon dioxide certainly accumulated, and probably to a greater degree in this series than in any of the others. With carbon dioxide accumulation, an increase in frequency of phrenic neurone discharge occurs from D to F, and in addition there is a recruitment of new units as is evident in E and more marked in F.

It is apparent that mechanisms for increasing frequency of discharge and recruitment of inspiratory motor units may be experimentally isolated from those governing rhythmicity and rate of respiration. With elimination of those inhibitory systems imparting rhythmicity to respiration, there remain inherent in the respiratory center of the lower part of the bulb, mechanisms for recruitment and increase of frequency of the final motor units in response to an increase of carbon dioxide in the inspired air.

DISCUSSION

The central respiratory mechanism may be divided for convenience into four systems: (i) the bulbar respiratory center-motor neurone system; (ii)

the vagal inhibitory system; (iii) the brain stem inhibitory system; and (iv) other excitatory and inhibitory systems.

1. The respiratory center-motor neurone system

Neurones of the inspiratory center making up the ventral part of the inferior reticular nucleus (21) are sensitive to changes in carbon dioxide tension of their fluid environment (8). Since the large residual and supplemental lung volumes effectively buffer the alveolar gas tension, and hence arterial blood, against any but slight tidal variations, the chemical stimulus acting upon these neurones may be considered essentially a constant one. In normal respiration, however, the response of these neurones to such a constant stimulus is phasic. Such phasic activity might result from processes inherent within the neurones of the respiratory center (3, 10, 31) or be imposed upon them by inhibitory mechanisms operating from without (18, 19, 29).

The present results are in accord with the latter thesis, for the combination of low transection of the brain stem and vagal section, eliminates such inhibitory mechanisms to a degree sufficient to convert phasic to maintained activity. Furthermore, they confirm Stella's contention (29) of the essential respiratory character of apneusis or inspiratory cramp in that they show, as did his results, that the magnitude of the cramp is related to the carbon dioxide content of the inspired air.

These studies, however, go further in showing that the inspiratory center-motor neurone system, in isolation, responds in a perfectly normal fashion to carbon dioxide stimulation. That is, the frequency of discharge and the numbers of final motor neurones responding, increase as the chemical stimulus is increased. But phasic interruption of this activity is lost and, as a consequence, the inspiratory pattern of slow augmentation and rapid decrement of discharge is not seen. However, this normal pattern may be restored by intermittent stimulation of the central end of one vagus. These results confirm our belief that inspiratory cramp is a specific response to elimination of inhibitory mechanisms, not a non-specific result of trauma of the section.

Studies of chemical activation of simple nervous structures, such as peripheral nerve and sympathetic ganglia, have shown that the individual units respond repetitively at frequencies proportional to the degree of disturbance of the chemical environment. Furthermore, additional units are recruited into activity as the degree of disturbance is increased (5). The similarity of the isolated respiratory center-motor neurone system to these simpler systems is sufficiently evident to require no further comment.

2. The vagal inhibitory system

Inspiration causes the lungs to expand, exciting stretch receptors of the parenchyma. These impulses, conducted centralward over the vagus nerves. lead to inhibition of phrenic nerve discharge (1, 16, 15). It is probable that these impulses, relayed through the nucleus solitarius, excite the dorsal reticular expiratory center (26). These expiratory neurones are functionally

interconnected with, and exert an inhibitory influence on the inspiratory center (23).

The magnitude of the inhibitory inflow over the vagi is proportional to the degree of lung stretch and hence to the depth of inspiration (1, 15). It is reasonable to assume that the ease with which the inspiratory center can be inhibited is inversely related to its level of excitation as revealed by the total respiratory effort. Thus with higher carbon dioxide tensions, inhibition is less easily effected and, as a consequence, deeper inspiration results. An increase in the frequency and number of active units brought about by the increased chemical stimulus produces an inspiration which attains a critical inhibitory depth sooner, *i.e.*, the slope of the inspiratory record is increased (Fig. 4, 5). The greater the level of excitation, the more rapid the recovery from inhibition and hence the shorter the expiratory pause.

Addition of the vagal inhibitory system to the respiratory center-motor neurone system imparts rhythmicity to respiration and provides the groundwork for increase of rate as well as depth of respiration.

3. The brain stem inhibitory system

The brain stem mechanism may be thought of as a subsidiary and possibly an adjuvant inhibitory system. It seems to operate in a manner parallel to the vagal inhibitory reflex system, but differs in that it lies wholly within the brain stem. Activity of the inspiratory center, relayed cephalically to higher levels, is returned caudally to the expiratory center along pathways in the ventro-lateral tegmentum of medulla and pons, leading to autoinhibition of inspiration (26). The slowing and deepening of respiration attendant on vagal section would seem to indicate that the brain stem inhibitory mechanism operates at a higher inspiratory level than the vagal reflex mechanism.

While we have no direct evident on the point, it would seem not unlikely that inhibition, which leads to the rapid decrement of frequency at the end of inspiration, persists through the expiratory pause and in diminishing degree into the start of the succeeding inspiration. The gradual augmentation of inspiratory discharge may be attributed in part to the gradual decay of inhibition.

4. Other excitatory and inhibitory systems

Afferents having origin in the various cranial nerves or in the sensory surface of the body and viscera impinge on either or both of the inspiratory and expiratory divisions of the respiratory center. Specific chemoreceptor afferents in the ninth and tenth cranial nerves play an important rôle in regulation of respiration under conditions of oxygen lack. Apparently the vagus afferents, described by Gesell and Moyer (14), which excite inspiration when the lungs are overdistended (15, 16), belong in this category also. In addition, cortical and hypothalamic connections modify respiration in important ways. Since our experiments have not dealt with these relationships, any discussion of their mode of action would be entirely speculative.

Summary

The central respiratory system may be functionally divided into four subsidiary systems: (i) the respiratory center-motor neurone system; (ii) the vagal inhibitory system; (iii) the brain stem inhibitory system; and (iv) other excitatory and inhibitory systems. The first three of these systems have been studied in isolation and in various combinations.

The respiratory center-motor neurone system regulates depth of inspiration by controlling motor unit impulse frequency and numbers of active units. The activity of this system in isolation is continuous and graded in degree in relation to carbon dioxide tension of the arterial blood.

The vagal inhibitory and brain stem inhibitory systems serve in a parallel manner to inhibit periodically the activities of the respiratory center-motor neurone system. Such periodic inhibition leads to rhythmic respiration and provides the groundwork for variation in rate.

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